USE OF ALUMINO-CALCIUM-PHOSPHOROUS-OXIDE (ALCAP) CERAMIC CAPSULES FOR DELIVERING POLYPEPTIDES OF VARIOUS MOLECULAR WEIGHTS

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ABSTRACT

USE OF ALUMINO-CALCIUM-PHOSPHOROUS-OXIDE
(ALCAP) CERAMIC CAPSULES
FOR DELIVERING
POLYPEPTIDES OF VARIOUS MOLECULAR WEIGHTS

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Ceramics composed of metallic oxides of calcium, phosphorous, aluminum and silicon in various combinations are biocompatible and have been used as prosthetics in animals as well as humans. Preliminary studies conducted by Khot, et al. (1980) established that ALCAP ceramics were capable of delivering insulin in a static system. The purpose of this investigation was to study the capability of ALCAP ceramics to deliver gamma globulin (M. W. 150,000), bovine serum albumin (M. W. 69,000) chymotrypsinogen (M. W. 26,000) and insulin (M. W. 5,766) in an in vitro continuous flow system over a period of seven days at 37°C.

Hollow ALCAP ceramic cylinders 2 cm long having outside and inside diameters of 1.3 and 0.5 cm respectively were fabricated from three different size (35-45, 45-60, and 60-75 um) calcined particles. Each ceramic capsule was sintered at 1425°C for 24 hours. The open ends of each ceramic capsule were sealed with a silicone rubber sealant. A 0.5 ml polypeptide solution having a concentration of 100 mg per ml was injected into each capsule by means of an 18 gauge needle.

The punctured end of the capsule was resealed to ensure the release of polypeptides through the ceramic pores.

Ceramics containing the polypeptides were placed in a continuous flow-through system chamber and a current of 0.2 ml per minute of phosphate buffered saline (pH 7.4) was perfused through the chamber at 37°C. The effluent was collected every 24 hours for seven days and the concentration of protein in the effluent was determined by the procedure of Lowry, et al. (1951). The entire investigation was conducted using triplicates of ceramics and aliquots. All the data collected were analyzed by means of analysis of variance at $P < .05$.

The release pattern of the four different substances was significantly influenced by the particle size of the ceramic capsules, the molecular weight of the polypeptide and the passage of time. None of the above parameters had a significant effect on the pH of the phosphate buffered saline effluent.

The ceramics studied are capable of delivering polypeptides of various molecular weights and can be used effectively as depots for delivering polypeptides in vivo.

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INTRODUCTION

Procedures for controlling the release of compounds have been recognized for many years. The concept of controlled release of chemicals was first used in the agricultural industry. To date farmers have reaped the benefit of releasing fertilizers slowly for more than thirty-five years. In the pharmaceutical field prolonged release of oral medications have been achieved by various techniques for more than thirty years.

The goal of the pharmaceutical industry is to achieve a minimum rate of drug delivery with maximum effects. This objective has been achieved to a certain extent with controlled release of certain kinds of drugs. However, delivery of polypeptide products cannot be achieved by these procedures since the enveloping procedure often requires chemical and thermal treatments.

Rejection of tissues capable of producing hormones has also been an insurmountable problem. However, attempts to reduce the immune response and increase the acceptance of such tissues are in progress.

A motorized portable insulin-delivery pump has been developed and used successfully in insulin-dependent dogs. This device is also undergoing clinical trials in some laboratories. Several implantable, miniaturized, self-contained, and self-powered osmotic pumps have

also been developed. However, the approval of latter device for human use is remote since they cannot be refilled in situ and will have to be recovered and replaced. The latter would naturally require repeated operative procedures and thus seems impractical.

Ceramics have been widely used for orthopaedic and dental implantation. The possibility of using aluminum based ceramic material as a means of controlled drug delivery system was recently advocated by Khot et al. (1980).

Molecular weights of different hormones and drugs vary widely. Therefore, whenever one thinks about developing a procedure for hormone or drug delivery, one should take into account both the needs of the patient and the characteristics of the needed hormone or drug.

This present investigation was designed to study the ability of the alumino-calcium-phosphorous oxide (ALCAP) ceramics to deliver gamma globulin (150,000 Daltons), bovine serum albumin (69,000 Daltons), chymotrypsinogen-A (26,000 Daltons) and insulin (5,766 Daltons) in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C. The main objective of these experiments was to show the general profile of delivery of each of these four polypeptides and also the range of molecular weight that can be delivered by ALCAP ceramics.

REVIEW OF LITERATURE

Controlled Drug Delivery

Man has yet to perfect drug delivery devices which can regulate the blood glucose levels of diabetics. According to Cahill, et al. (1976) and Ingelfinger (1977) there is a strong relationship between the regulation of blood glucose and the development of long-term microangiopathic and neuropathic complications in diabetes mellitus. Even under optimal conditions, conventional treatment with insulin is associated with wide swings in plasma glucose well outside the normal range (Service, et al. 1970). Treatment of juvenile-onset diabetes with multiple injections of rapid-acting or intermediate-acting insulin are associated with persistence of hyperglycemia (plasma glucose in excess of 200 mg %) or the development of hypoglycemia (plasma glucose less than 50 mg %) or both (Service, et al. 1970, and Molnar, et al. 1972). In contrast the plasma glucose concentration in healthy subjects on their usual diet, generally does not vary more than 40-60 mg % and remains between 70-120 mg % when monitored over a duration of 24 hours (Service, et al. 1970, and Tasaka, et al. 1975).

Implantable Delivery Devices

The blood glucose regulation was markedly improved for a period of 12 to 24 hours with the use of an artificial pancreas (Albisser, et al. 1974, and Mirouze, et al. 1977). This device provided the maintenance of the blood glucose concentration within a desired range by the continuous intravenous infusion of insulin at a variable rate. Regulation of the insulin-infusion rate on a moment-to-moment basis was established by on-line monitoring of the blood glucose and computerized feedback signal (Albisser, et al. 1974). However the use of such a device is limited by the lack of an implantable glucose sensor and the need for continuous blood withdrawal and the attachment of the patient to a glucose analyzer. Genuth, et al. (1977) and Service (1978) reported an improved blood glucose regulation without feedback control of insulin-delivery rates by increasing the amount of insulin delivery before each meal. However, infection and thrombosis are always inherent with the use of any long-term intravenous delivery system (Tamborlane, et al. 1979).

Pickup, et al. (1978) employed a pre-programmed insulin-delivery system in which insulin was administrated via the subcutaneous route by means of a portable infusion pump. However this device can deliver insulin only on a fixed basis (8X of the basal rate). Reduction in mean blood glucose concentration was achieved in only six of fourteen patients by the use of this device and two patients exhibited serious

problems in regulating blood levels of glucose (Pickup, et al. 1978). Recently Tamborlane, et al. (1979) have used a subcutaneous portable, battery-powered infusion system to deliver insulin. By means of this device they were able to adjust delivery of insulin at different pre-meal insulin and basal infusion rates. While most of the pre-programmed insulin-delivery systems have been studied in adult subjects (Genuth, et al. 1977 and Pickup, et al. 1978) the delivery of insulin by Tamborlane, et al.'s (1979) device was studied in children and adolescents. The regulation of blood glucose level in this age group has always been a difficult task and is associated with higher risks of long-term microvascular complications (Cahill, et al. 1976). Since the drug is delivered by means of a 27-gauge needle inserted in the subcutaneous tissue the toxic effects of metal corrosion cannot be ignored. The device cannot be used by everyone since the patient has to be educated to use the computer-controlled delivery system. Since insulin is destroyed selectively in subcutaneous tissue (Duckworth and Kitabchi, 1981), the system will also need constant upgrading to overcome the increase in enzyme activity. However at present the future of this device looks quite good for a select group of individuals.

Osmotic Pump Delivery Systems

Alza Corporation (Palo Alto, California, 94303) has developed small self-powered, continuous-delivery infusion pumps of different

kinds for in vivo or in vitro use in the laboratory. These pumps also do not require external connections (Yates, 1978). According to Eckenhoff, et al. (1976) an osmotic minipump (Alzet Osmotic Minipump) is a cylinderical device (25 mm in length and 6.5 mm in diameter) for fluid release which can be implanted subcutaneously or interaperitoneally in animals as small as the mouse, for parenteral administration of bioactive agents at constant rates for periods up to 7 days. The external surface of the minipump is composed of a rate-controlling semi-permeable membrane. The inside deformable drug reservoir is coated with an osmotic agent. Since the semipermeable membrane is made of cellulose it is biocompatible. When a minipump is placed within a living animal, water is pulled osmotically into the reservoir by the osmotic agent at a rate controlled by the membrane. Since the membrane is rigid, the entrance of water displaces the material from the reservoir, which collapses gradually with time, pumping the contents out of the minipump. The 170 microliter reservoir is filled by the user. The reservoir is compatible with water, physiogical salt solutions, polyethylene glycols, propylene glycol, and with dilute aqueous ethanol. The duration of delivery depends on the type of membrane. This implanted minipump can also be connected to a small catheter for systemic delivery or administration of agents intravenously, intra cisternally, or tissue sites.

The use of Alzet osmotic minipump in laboratory research started in the early 1977. The minipump was mainly designed for use in small laboratory animals, but by simultaneously implanting multiple pumps an investigator may achieve adequate mass fluxes in dogs, monkeys, baboons, sheep, goats, or other large animals. For example normal blood sugar levels have been successfully maintained in a severely diabetic dogs weighing 33 kilograms, by means of four minipumps each delivering 0.25 U of insulin per hour subcutaneously (Yates, 1979).

Minipumps have usually been implanted subcutaneously and less commonly intraperitoneally. Infusion of tissues has also been attained with the aid of a minipump fitted with a catheter flow moderator. Infusion of various fluids and drugs in the ventricular system of the brain, lumen of the uterus, fornix of the eye, corneal tissue, venous system, tissue of the visual cortex, and cerebspinal fluid system have been achieved by the use of the minipumps fitted with catheters (Benson, 1979).

Siew and Goldstein (1976)used the Alzet Osmotic Minipump to administer sodium barbital to mice at a constant rate of about 0.5 mg per hour for studying the possibility of rapid development of barbiturate tolerance and physical dependence. They concluded that the osmotic pumps may be useful in studying the mechanisms of barbiturate tolerance and dependence.

Alzet Osmotic Minipumps have also been used to study the effects of prolonged continuous low rate delivery of antigen on primary and secondary immune responses (Amkraut, et al. 1976). Martins, and Amkraut (1978) immunized mice with tetanus toxoid antigen mixed with graded doses of lipopolysaccharides using an osmotic minipump and subcutaneously by means of single injection of the mixture. Results of their study showed that tetanus toxoid in same doses was significantly more immunogenic when delivered continuously by means of a minipump. The data obtained by these workers are important in the sense that they show effective utilization of the pump in immunizing animals. However, it also points out the fact that the same phenomenon can occur when some other biological agents capable of eliciting an immune response are administered by means of an osmotic pump.

Osmotic minipumps have also been used for infusing thyrotropin-releasing hormone (TRH) over a period of five days. These studies were conducted to investigate the effects of TRH on secretation of prolactin and growth hormone from the pituitary. The experiment was conducted in ovariectomized ewes and the minipumps implanted subcutaneously succeeded in providing a sustained release of 500 ug of hormone per day (Klindt, et al., 1979).

An osmotic minipump filled with 170 ul of an aqueous sodium pork insulin solution of 3200 U/ml in 1.6% glycertol (pH 7.4) has also been placed in a subcutaneous tunnel in the posterior aspect of the upper leg

of rhesus monkey fetus. In this position the minipump was reported to deliver 19 U or 6 ul of insulin per day continuously for 28 days (Susa, et al. 1979).

McDonald, et al. (1980) used the Alzet osmotic minipump to deliver a test solution to the brain of rats by means of PE 60 tubing connected to the minipump cannula. The osmotic pump was inserted between the two scapulas. The test solution by this technique can be delivered at a constant rate of 1 ul/h ± 15% (Wei and Loh, 1976). Continuous infusion of angiotensin II at the rate of 50 ng/min has been attained by implanting the osmotic minipump in rats. This implantation procedure has been employed to study the effects of increased levels of circulating angiotensin II upon the steroidogenic activity of the adrenal glomerulosa cells. Prolonged infusion of angiotensin II in normal and hypophysectomized rats was found to reduce the effects of sodium restriction on angiotensin II receptors and enzymes of the aldosterone biosynthetic pathway (Aguilera, et al., 1980).

After dissolving a converting enzyme inhibitor (SQ 14,225) of angiotensin I to angiotensin II in phosphate buffered saline containing 0.5 mM ascorbic acid, Schirar, et al. (1980) administered the solution by means of an intraperitoneally implanted osmotic minipump at the rate of 1 ug/min in pregnant rats. Angiotensin II concentrations did not change between day 4 and 9 of pregnancy in the sterile horns and indicated that the plasma angiotensin II levels did not play a role in the non-gravid uterine horns.

Harwood, et al. (1980) implanted Alzet osmotic minipumps in the peritoneal cavities of female gonadotropin-primed rats to infuse gonadotropin-releasing hormone (GnRH) agonist analogs in order to determine whether the immature ovary contained gonadotropin-releasing hormone receptors before gonadotropin priming. They also wanted to know whether gonadotropin-releasing hormone was capable of antogonizing the effect of exogenous pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (HCG) during the maturation process of the ovaries. Results of their investigation showed that the administration of GnRH agonist analogs completely inhibited the trophic actions of both gonadotropins. The GnRH induced a decrease in luteinizing hormone (LH) and prolactin (PRL) receptors in hypophysectomized animals indicating that GnRH agonist analogs can act directly on the ovary and that their effects are not only due to the secondary release of gonadotropins from the pituitary gland.

Mode, et al. (1981) used Alzet osmotic minipumps for continuous administration of human growth hormone (hGH) at the rate of 5 ug/h for seven days in normal male rats under varying conditions to study the metabolism of 4-(4-^{14}C) androstene -3-17-dione in the microsomal fraction of rat livers. They also used the pumps for continuous infusion of prolactin and growth hormone to study the effects of these homologous hormones on the hepatic steroid metabolism. Both hormones were infused continuously at the rate of 10 ug/h for

seven days, to increase the serum concentrations of the hormones to normal levels in hypophysectomized animals. Human growth hormone was found to cause a complete feminization of the hepatic steroid metabolism (i.e., it increased the 5α- reductase and decreased the 6β- and 16- α - hydroxylase activities). The feminizing capacity of the human growth hormone was affected neither by hypophysectomy and castration nor by adrenalectomy and thyroidectomy of male rats, indicating that the adrenal and thyroid gland are not involved in the mediation of the feminizing effect of the human growth hormone. The same dose of human growth hormone also refeminized the liver steroid metabolism in hypophysectomized-ovariectomized female rats. Rat growth hormone caused a partial feminization of the hepatic steroid metabolism in hypophysectomized male animals, rat-PRL did not cause feminization of these rats. The results of these studies show that growth hormone or growth hormone related hormones are involved in sexual differentiation of liver steroid metabolism (Mode, et al. 1981).

The administration of ovine-PRL or bovine growth hormone as well as a combination of both hormones by means of an Alzet osmotic minipump has been reported by Norsted, et al. (1981). According to them administration of growth hormone by these procedures induces the development of estrogen receptors in the livers of dexamethasone-treated hypophysectomized rats. However complete restoration of hepatic estrogen receptor levels was not achieved by administration of rat-PRL by osmotic minipumps (Norsted, et al. 1981).

Polymer Delivery Systems

Hormonal contraceptive therapy requires that the drug be administered orally or intramuscularly. These two methods usually have certain disadvantages. The daily intake of the tablets in the oral method can result in harmful side effects, such as cancer. Although the intramuscular administration of the contraceptive drugs (steroids) prevents conception for extended periods it cannot be reversed quickly (Roseman, 1974).

In order to overcome the side effects usually associated with the oral administration of contraceptive steroids, a biodegradable drug delivery system was developed by Sidman, et al. (1976). They encapsulated steroids in glutamic acid and leucine co-polymers. The objective of this work was to fabricate an injectable biodegradable drug reservoir that would provide a constant, controlled drug release of contraceptives for 6 to 12 months. In one of the two approaches used in this study approximately 40 to 60% progesterone was dispersed in a sintered network structure of the polymer. After providing an initial peak of progesterone the rod-shaped capsular implants released the hormone in a consistent manner for more than 100 days in rats. Rods of smaller size showed a more consistent progesterone release pattern than rods of larger dimensions. Rate of progesterone release after the initial peak dropped significantly on implantation of larger size rods.

Pitt, et al. (1976) characterized homo and copolymers of glycoside, DL-Lactide, E-caprolactone, DL-E-decalactone and other monomeric and dimeric lactones for developing a new biodegradable polymer subdermal steroid release system. They reported that the biodegradation of films and capsules of these polymers was not dependent on the chemical structure but rather on the molecular weight and morphology of the polymer.

Nuwayser, et al. (1976) developed a procedure for microencapsulating progesterone in a bioresorbable homopolymer of L(-) lactide. According to them the microcapsules when injected in a suspension phase should be absorbed by the host after the completion of drug delivery. The goal of these investigations was to provide semipermeable capsule reservoirs for delivering progesterone at a constant rate for a period of one year. The microencapsulation was done either by the process of coocervation, coagulation or air suspension coating. Injection of progesterone, encapsulated by the above procedure, in rabbits resulted in an initial rapid delivery of the hormone and was followed by a sustained release of the hormone for about 90 days.

Prostaglandins have been used in the third trimester to terminate pregnancies. However large doses of prostaglandin-F (PGF_2) have serious side effects. Hence Davis and Chang (1972) studied the feasibility of administering PGF_2 by means of polyacrylamide and silastic tubes.

According to Davis and Chang (1972) polyacrylamide polymer allowed better delivery of PGF_2 in the surrounding media when tested in an in-vitro testing system. They also reported a significant decrease in pregnancy on implanation of the PGF2 polyacrylamide reservoirs in the vagina of hamsters two days after mating. Remating of these hamsters after the withdrawal of the PGF_2 polyacrylamide reservoirs resulted in restoration of the fertility and pregnancy.

Davis (1974) also observed absorption of various radioactively labelled substances from cylindrical polyacrylamide and polyvinylpyrolidone implants. They observed the release of bovine serum albumin, rabbit immunoglobulin, bovine pancreatic insulin and rat luteinizing hormone, $Na^{125}I$ and (3H) prostaglandin F2 (PGF_2) from the polyacrylamide and polyvinylamide reservoirs. The absorption rate of each of the above solutes (in vivo) was used to determine its diffusion coefficient. The diffusion coefficient of the enclosed material showed a logarithmic relationship between the concentration of the cross-linked polymer gels and the molecular weight of each solute. The in vivo relationship between the delivery rate of the substance and the polymer was later confirmed by in-vitro studies.

Silicone Rubber Delivery Systems

In the last two decades contraceptive steroids have been delivered by means of silicone rubber (polydimethylsiloxane) devices (Roseman, 1974).

The importance of implantation of drugs in capsules of silastic (polydimethylsiloxane) and their prolonged local or systemic administration was realized first in 1964 by Folkman and Long. A wide range of application including use in experimental medicine, treatment of chronic diseases, prophylaxis of infectious diseases or allergies was advocated by Folkman and Long (1964).

The most significant application of silicone rubber involved the diffusion of anesthetic and analgesic drugs (Folkman, et al., 1966). Folkman and Mark (1968) were able to induce anesthesia and analgesia in dogs by passing the vapors of either nitrous oxide, ether, halothane, or cyclopropane, through a coil of silicone rubber tubing, each end of which was placed in an artery or a vein.

The encapsulation of the antimalarial agent, Pyrimethamine (Daraprim) in silastic polymer and its implantation in chicks was reported by Powers in 1965. According to Powers (1965) implanation of these slow releasing capsules containing Daraprim protected the chicks from malaria.

In the field of contraception, Roseman (1974) used silicone rubber as a matrix for the controlled release of contraceptive steroids. However, Roseman was more concerned about the mechanisms of the drug delivery from the silicone rubber and the factors affecting these mechanisms. His data showed that the structure of the steroid affected the diffusion of the steroid from the silicone rubber matrices or across

the rubber membranes. He also noticed that transport rates were dependent upon the degree of lipophilicity of the released steroid and the thickness of the silicone rubber membrane. Those steroids with higher silicone solubility generally exhibit faster release rates.

Silicone rubber has also been used for slow release of atropine and histamine. Bass, et al. (1965) were able to induce longer mydriasis (pupil dilation) by means of subcutaneous implants of atropine encased in silastic tubes. They were also able to induce gastrointestinal lesions in dogs by implanting silastic tubes of histamine at weekly intervals.

Four different formulations of silicone rubber vaginal contraceptive devices were designed, fabricated and tested for the release of controlled doses of three different progestogens: progesterone, norethindrone (two dose levels), and d-norgestrel, in 90 day clinical trials by Burton, et al. (1979). The data obtained with the use of 70 devices showed that in-vivo release rate from these devices for progesterone was 1400 $\pm$ 30 U; norethindrone, 49.4 $\pm$ 2.4 U; and d-norgestrel, 26.6 $\pm$ 1.4 U. Use of 3 steroid filled silicone rubber devices resulted in normal ovulation but inhibition of penetration of the cervix by spermatozoa. Silicone rubber devices filled with high doses of norgesterel also inhibited ovulation.

Ceramic Implant Delivery Systems

The use of ceramics as a biomaterial dates back to 1892. According to Peltier (1961) plaster of Paris was used by Dressman in 1892 to correct bone defects. Various forms of biocompatible and non-toxic ceramics are in use today. Both resorbable and non-resorbable ceramics have been used as biomaterials to correct defects in the skeletal system (Hulbert, et al. 1970, 71; Graves, et al. 1971; Young, 1973; Graves and Noyes, 1974; Graves, et al. 1975 and Mattie, et al. 1981). Initially Graves, et al. (1971) prepared both single-phase and polyphasic calcium aluminate ceramic implants. After thirty-two weeks of implanation they observed complete impregnation of the polyphasic ceramic implants with connective tissue and comparatively no alteration of the single-phase ceramic material. Graves and Noyes (1974) experimented with calcium aluminate ceramics containing 5 to 20% phosphorous pentoxide (P_2O_5). They reported that the amount of phosphorous pentoxide incorporated in the calcium aluminate ceramics significantly influenced the surface characteristics and microstructure of the unimplanted ceramics and resorption rate of the ceramics implanted in Rhesus monkeys. Toxic, allergic, or carcinogenic responses were not observed for 2.5 to 3 years of investigation in any of the animals.

Biochemical, radiographic, scanning-electron-micrographic, and histological data obtained on calcium aluminate ceramics containing

P_2O_5 suggested that ceramics containing 20% P_2O_5 were ideal for correcting bone defects in rats as well as monkeys (Bajpai, et al. (1976).

Although ceramics have been widely used for orthopaedic and dental implantation their use as drug delivery devices has not received much attention (Khot, et al., 1980). Khot (1979) fabricated ceramic cylinders of different porosity and density from a mixture of aluminum, calcium and phosphorous oxide powders (ALCAP), and used them to deliver insulin (in-vitro) in a static system of phosphate buffered saline at a physiological pH (7.4) and temperature (37°C). The results of this study showed that the insulin release was affected by the elution time, starting particle size, and sintering temperature of the ceramics. Sintering time of the ceramic material alone did not affect the delivery of insulin. Thus the need for further characterization of ALCAP ceramic reservoirs is quite obvious.

MATERIALS AND METHODS

The study conducted by Khot (1979) on ALCAP ceramics showed that these ceramics were capable of delivering insulin in a phosphate-buffered-saline (pH 7.4) static system at 37°C. The delivery rate of insulin monitored by Khot, et al. (1980) was not affected by the sintering time of the ceramics. However, the calcined particle size of the ceramics and the elution time had significant effects on the delivery of the insulin in the static system. Hence it was considered necessary to reinvestigate the effect of ceramic particle size on the delivery profile of polypeptides in a continuous flow-through system. Thus for this investigation ceramics were fabricated from calcined particle sizes of 35-45, 45-60, and 60-75 um by the Department of Material Science of the University of Dayton using the procedure of Khot, et al. (1980) and Mattie, et al.(1981) (appendix 6). The ceramics were then used for delivering gamma globulin (150,000 Daltons), bovine serum albumin (69,000 Daltons), chymotrypsinogen-A (26,000 Daltons), and insulin (5,766 Daltons) in an in-vitro continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C. The delivery of the polypeptides and proteins by the ceramic capsules was monitored for seven days and the effluent was collected every twenty-four hours. Aliquots from each

day's collection were tested for the presence of proteins in the effluents by a colorimetric procedure (appendix 5) published by Lowry, et al. (1951). The data collected were analyzed by means of analysis of variance at a significant level of $P < 0.05$ (Sokal, 1969).

Preparation of Ceramics

Twelve hollow ALCAP ceramic cylinders 2 cm long having an outside diameter of 1.3 Cm and inside diameter of 0.5 cm were fabricated from each of the three ranges of calcined particle size (35-45, 45-60, and 60-75 um) by the procedure of Khot, et al. (1980) and Mattie, et al. (1981) (appendix 6). Each ceramic capsule was sintered at 1425°C (2597°F) for 24 hours. Triplicates of ceramics from each range of calcined particle size group were used to deliver each of the four polypeptides or proteins. For example three ceramics from the 45-60 um particle size group were tested for delivering gamma globulin (gG).

Each ceramic cylinder was sealed at both ends with silicone rubber sealant (Dow Corning), 24 hours before conducting the experiment. To avoid denaturation of the polypeptide by heat generated due to the reaction between water and the ceramic materials, one ml of phosphate buffered saline (PBS) was injected in the ceramic cavity using an 18G needle to saturate the ceramic material. A few minutes later, a 0.5 ml polypeptide solution having a concentration of 100 milligrams per milliliter was injected in each capsule. The punctured end of the capsule

was resealed immediately after the injection of the polypeptide solution (suspension) with the silicone rubber sealant to ensure the release of the polypeptide through the ceramic pores and not through the punctured end. Although the sealant needs only five minutes to cure it was allowed ten minutes to "skin off" before the capsule was placed in the continuous flow-through system (Fig. 1).

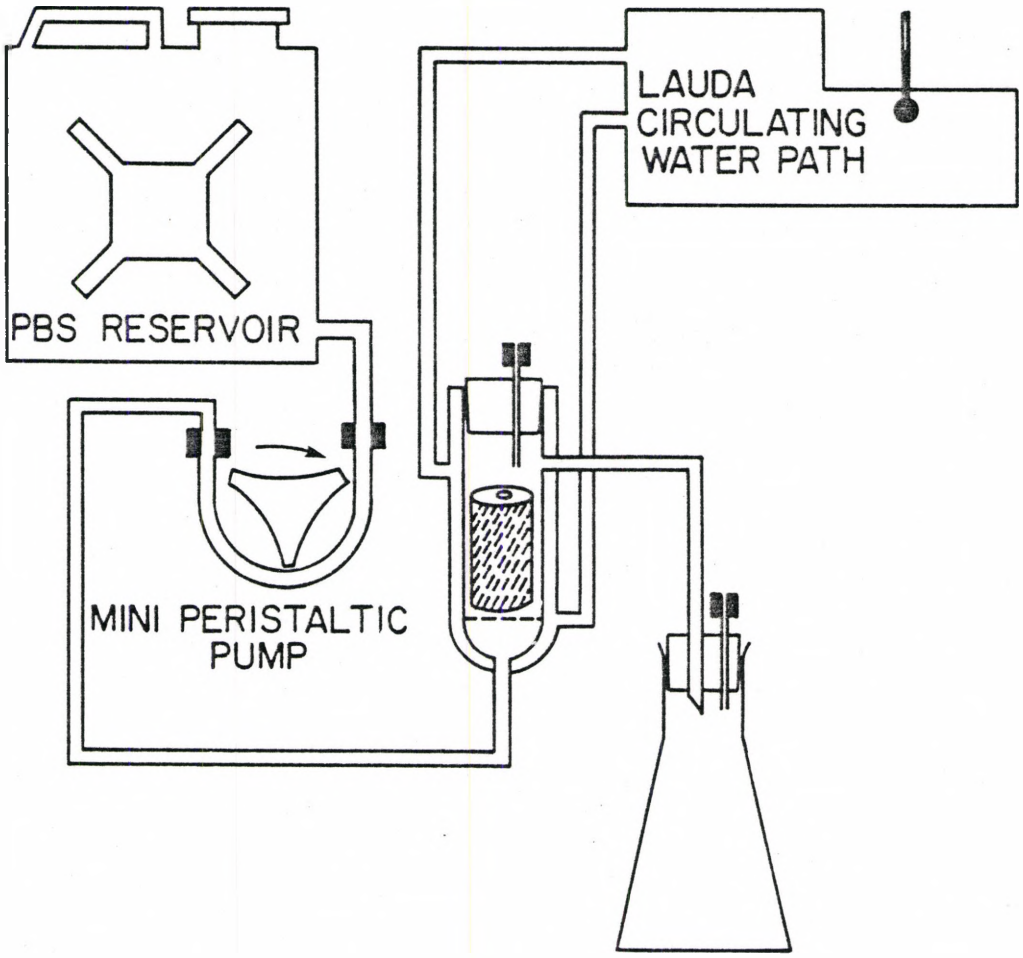
Continuous Flow-Through System:

A circulating water bath, flow-through chamber, peristaltic pump, PBS reservoir, and collection flasks were assembled into a continuous flow-through system (Fig. 1).

Chamber:

The chamber is composed of a cylindrical glass cavity (1.5 cm diameter and 5.5 cm long) and has three openings. Phosphate buffered saline enters the main chamber from the bottom opening. The ceramic capsule containing the polypeptide is placed in the main chamber via the top opening and it rests on a porous glass disc. The top opening was later closed by a rubber stopper to prevent evaporation of the fluid in the chamber. An 18G needle was inserted through the rubber stopper to equilibrate the chamber pressure with the atmospheric pressure. The effluent was collected from the side opening. The entire chamber was jacketed by means of a water jacket. Water from the circulating water bath was pumped through the water jacket to maintain the temperature of the main chamber at 37°C.

Figure 1: A diagramatic representation of the continuous flow-through system. Basically it consists of a buffer reservoir, a peristaltic pump, glass jacket around the main chamber for maintaining the temperature at 37°C by means of a circulating water bath (the ceramic rests on a porous glass disc in the main chamber) and the collecting flask.


LAUDA
CIRCULATING
WATER PATH
PBS RESERVOIR
MINI PERISTALTIC
PUMP

Collecting Flasks:

Three hundred mililiter Pyrex flasks (Pyrex No. 4980) were used for collecting the effluents. Each collecting flask was stoppered by means of a rubber stopper and connected to the side opening of the main chamber by means of a polyethylene tubing. The atmospheric pressure of the flask was maintained in the same manner as in the main chamber.

Phosphate Buffered Saline (PBS) Reservoir:

A twenty liter plastic tank containing the PBS solution (appendix 5) was connected to a Mini Peristaltic Pump (Markson Science, Inc., Del Mar, Ca. 92014). The outlet of the peristaltic pump was connected to the bottom opening of the main chamber by means polyethylene tubing. A current of PBS was pumped past the ceramic by means of the peristaltic pump at the rate of 0.2 ml/min.

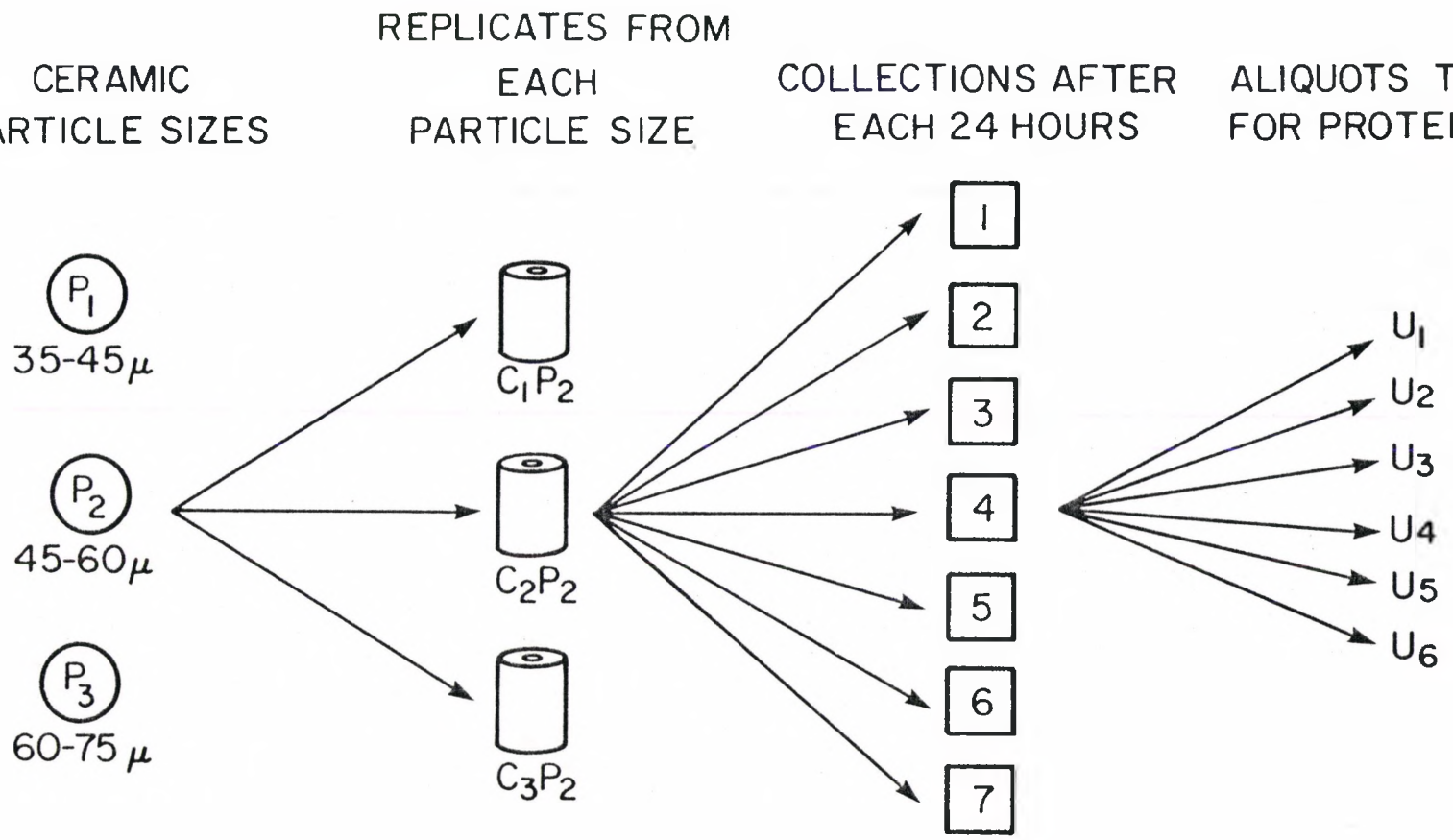
Circulating Water Bath:

A "Lauda Thermostat" circulating water bath (Messgerate-Werk-Lauda, Western Germany) was connected to the water jacket of the chamber to maintain the temperature at 37°C.

Experimental Procedures:

After the three ceramic capsules containing the polypeptide were placed in the three chambers, the effluents were collected and the flasks were changed every twenty four hours for a total period of seven days.

Figure 2: Illustration of the sampling procedure. Three ALCAP ceramics fabricated from each particle size were used to deliver each polypeptide for 7 days and 6 aliquots from each day's collection were tested for the concentration of proteins.


CERAMIC PARTICLE SIZES
REPLICATES FROM EACH PARTICLE SIZE
COLLECTIONS AFTER EACH 24 HOURS
ALIQUOTS TESTED FOR PROTEIN CONC.
P1
35-45μ
P2
45-60μ
P3
60-75 μ
C1P2
C2P2
C3P2
1
2
3
4
5
6
7
U1
U2
U3
U4
U5
U6

Six aliquots of 1 ml each from each day's collection were analyzed by the procedure of Lowry, et al. (1951) for the presence of proteins and polypeptides (appendix 5). Known amounts (0-350 mg) of bovine serum albumin (BSA) were used to prepare the standard curve. A "STAT 10" computer program (University of Dayton, Computer Library 1981) was used to obtain linear regression and corrected standard curve from replicates of BSA standards. A computer program using the corrected standard curve was written to convert the optical density readings to miligrams of polypeptide or protein per mililiter of solution. The total amount of polypeptide for each day's collection was determined by multiplying the miligrams of polypeptide per mililiter of solution by the total amount of effluent (ml) collected (0.2 ml x 1440 min (1 day) = 288 ml/day).

Measurement of pH:

A 5 ml aliquot was saved from each day's collection for future studies and the rest of the effluent was used for determination of pH. Changes in pH were measured in triplicates from each day's collection by means of a digital ion analyzer (Model No. 801A, Orion Research, Inc., Cambridge, Massachusetts 02139).

Data Analysis:

All data collected were analyzed by means of an "AOV" analysis of variance computer program (University of Dayton, Computer Library,

1981). The effects of the following variables on the release of the polypeptide from the ceramics were analyzed: (1) elution time in days, (2) particle size of the ceramics (35-45, 45-60, and 60-75 um), and (3) ceramic implants. Significant differences between means were analyzed at $P<0.05$ level using the Student Newman Keuls multiple range test (appendixes 1, 2, 3, and 4).

RESULTS

Delivery of Gamma Globulin:

Elution pattern of gamma globulin by the three particle size of ALCAP ceramics has been tabulated in table 1 and presented in Figure 3. Statistical analysis of the data shows that the delivery rate of gamma globulin by means of ALCAP ceramics was significantly influenced by the passage of time as well as the particle size of the ceramics. Differences in the amounts of gamma globulin delivered by ceramic replicates were not significant (appendix 1). The delivery rate of gamma globulin was inversely proportional to the particle size of the ceramic during the first two days of elution. On the third and fourth day the highest amount of gamma globulin was eluted from the ceramics fabricated from the medium-size particles. The amount of gamma globulin eluted from the ceramics fabricated from the small and large size particles corresponded to the particle size of the ceramics. During the last three days the amount of gamma globulin delivered by the ceramics fabricated from small and medium size particles were not significantly different. Ceramics fabricated from the largest particle size delivered the highest amount of gamma globulin.

Delivery of Bovine Serum Albumin:

Data obtained from the experiments conducted on the delivery of bovine serum albumin by the three different particle size ALCAP ceramic capsules are presented in table 2 and figure 4.

TABLE 1. MILLIGRAMS* OF GAMMA GLOBULIN RELEASED DAILY (FOR SEVEN DAYS) IN PHOSPHATE BUFFERED SALINE (pH 7.4) EFFLUENT FROM ALCAP CERAMIC CAPSULES IN A CONTINUOUS FLOW-THROUGH SYSTEM AT 37°C.

PARTICLE SIZE (μm)	AMOUNT OF GAMMA GLOBULIN (mg)							
	DAY (1)	DAY (2)	DAY (3)	DAY (4)	DAY (5)	DAY (6)	DAY (7)	TOTAL
60-75	10.4 ± 0.3 AI	13.5 ± 0.3 BI	8.7 ± 0.2 CI	5.4 ± 0.3 DI	4.4 ± 0.4 EI	3.3 ± 0.3 FI	1.5 ± 0.2 GI	47.2 ± 0.5
45-60	15.3 ± 0.3 AII	15.3 ± 0.3 BII	9.5 ± 0.3 CII	5.7 ± 0.2 DII	1.6 ± 0.3 EII	0.5 ± 0.3 FII	0.4 ± 0.2 FII	48.3 ± 0.7
35-45	17.5 ± 0.3 AIII	16.5 ± 0.3 BIII	6.5 ± 0.3 CIII	3.5 ± 0.3 DIII	1.6 ± 0.2 EII	0.3 ± 0.2 FII	0.2 ± 0.1 FII	46.1 ± 0.7

*ALL DATA PRESENTED AS MEAN ± STANDARD DEVIATION

Mean ± S.D. values followed by different letters for each particle size (horizontally) are significant from each other at $P < 0.05$

Mean ± S.D. values followed by different Roman numerals for each day (vertically) are significant from each other at $P < 0.05$

Figure 3: The effect of particle size on the delivery profile of gamma globulin by means of ALCAP ceramic capsules in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.

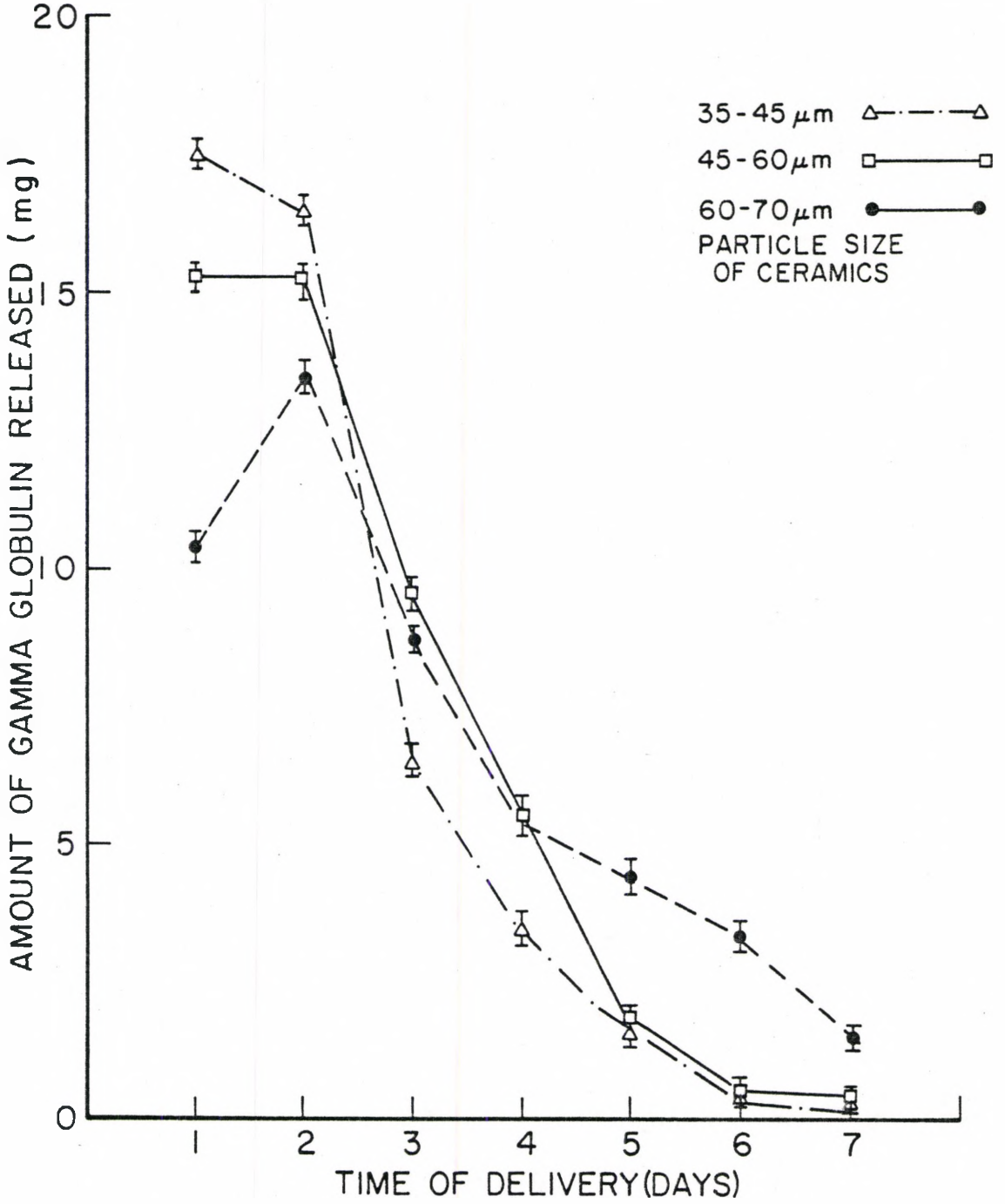

20
15
10
5
0
AMOUNT OF GAMMA GLOBULIN RELEASED (mg)
1
2
3
4
5
6
7
TIME OF DELIVERY (DAYS)
35-45 μm
45-60 μm
60-70 μm
PARTICLE SIZE
OF CERAMICS

TABLE 2. MILLIGRAMS* OF BOVINE SERUM ALBUMIN RELEASED DAILY (FOR SEVEN DAYS) IN PHOSPHATE BUFFERED SALINE (pH 7.4) EFFLUENT FROM ALCAP CERAMIC CAPSULES IN A CONTINUOUS FLOW-THROUGH SYSTEM AT 37°C.

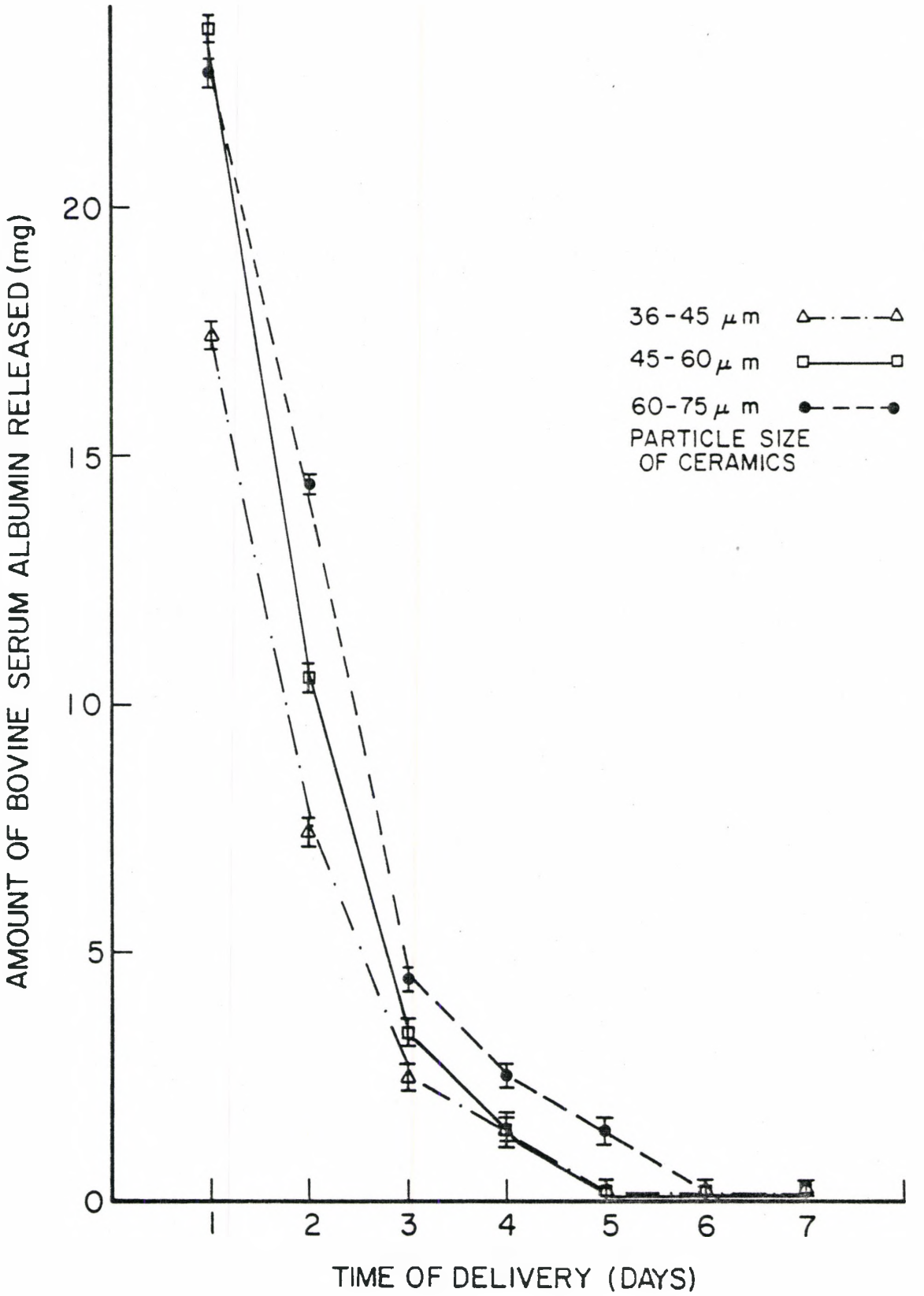
PARTICLE SIZE (μm)	AMOUNT OF BOVINE SERUM ALBUMIN (mg)							
	DAY (1)	DAY (2)	DAY (3)	DAY (4)	DAY (5)	DAY (6)	DAY (7)	TOTAL
60-75	22.6 ± 0.3 A I	14.4 ± 0.2 BI	4.5 ± 0.3 CI	2 5 ± 0.3 DI	1.4 ± 0.3 EI	0.2 ± 0.0 FI	0.1 ± 0.0 GI	45.7 ± 0.9
45-60	23.5 ± 0.3 AII	10.5 ± 0.3 BII	3.4 ± 0.3 CII	1.4 ± 0.3 DII	0.2 ± 0.1 EII	0.2 ± 0.0 EI	0.2 ± 0.0 EII	39.4 ± 0.5
35-45	17.4 ± 0.3 AIII	7.4 ± 0.3 BIII	2.5 ± 0.3 CIII	1.5 ± 0.3 DII	0.2 ± 0.0 EII	0.2 ± 0.0 EI	0.1 ± 0.0 FI	29.3 ± 0.6

*ALL DATA PRESENTED AS MEAN ± STANDARD DEVIATION

Mean ± S.D. values followed by different letters for each particle size (horizontally) are significant from each other at $P < 0.05$

Mean ± S.D. values followed by different Roman numerals for each day (vertically) are significant from each other at $P < 0.05$

Figure 4: The effect of particle size on the delivery profile of bovine serum albumin by means of ALCAP ceramic capsules in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.


AMOUNT OF BOVINE SERUM ALBUMIN RELEASED (mg)
0
5
10
15
20
1
2
3
4
5
6
7
TIME OF DELIVERY (DAYS)
36-45 μm
45-60 μm
60-75 μm
PARTICLE SIZE
OF CERAMICS

Statistical analysis of the data showed that the elution rate of bovine serum albumin was significantly influenced by the passage of time and particle size of the ceramics. During the first three days of elution the amount of BSA delivered was directly proportional to the ceramic particle size. The amount of BSA eluted during the next two days from ceramics fabricated from the small and medium size particles were not significantly different. Ceramics fabricated from the largest particle size delivered the highest amount of BSA on day four and five. Particle size of the ceramic did not influence the delivery of BSA on the sixth day of elution. Ceramics fabricated from medium particle size showed the highest rate of BSA delivery on the last day of the experimental trial. Differences between the amount of BSA delivered by ceramics fabricated from small and large size particles were not significant. Amount of BSA delivered from replicates were not significant (appendix 2)

Delivery of Chymotrypsinogen-A:

Milligrams of chymotrypsinogen-A delivered by ALCAP ceramics fabricated from three different particle sizes for a period of seven days are shown in table 3, and figure 5.

Analysis of variance and range test of the data showed that both particle size of the ceramics and the elution time have a significant effect on the delivery rate of chymotrypsinogen-A from the ALCAP ceramics.

TABLE 3. MILLIGRAMS* OF CHYMOTRYPSINOGEN-A RELEASED DAILY (FOR SEVEN DAYS) IN PHOSPHATE BUFFERED SALINE (pH 7.4) EFFLUENT FROM ALCAP CERAMIC CAPSULES IN CONTINUOUS FLOW-THROUGH SYSTEM AT 37°C.

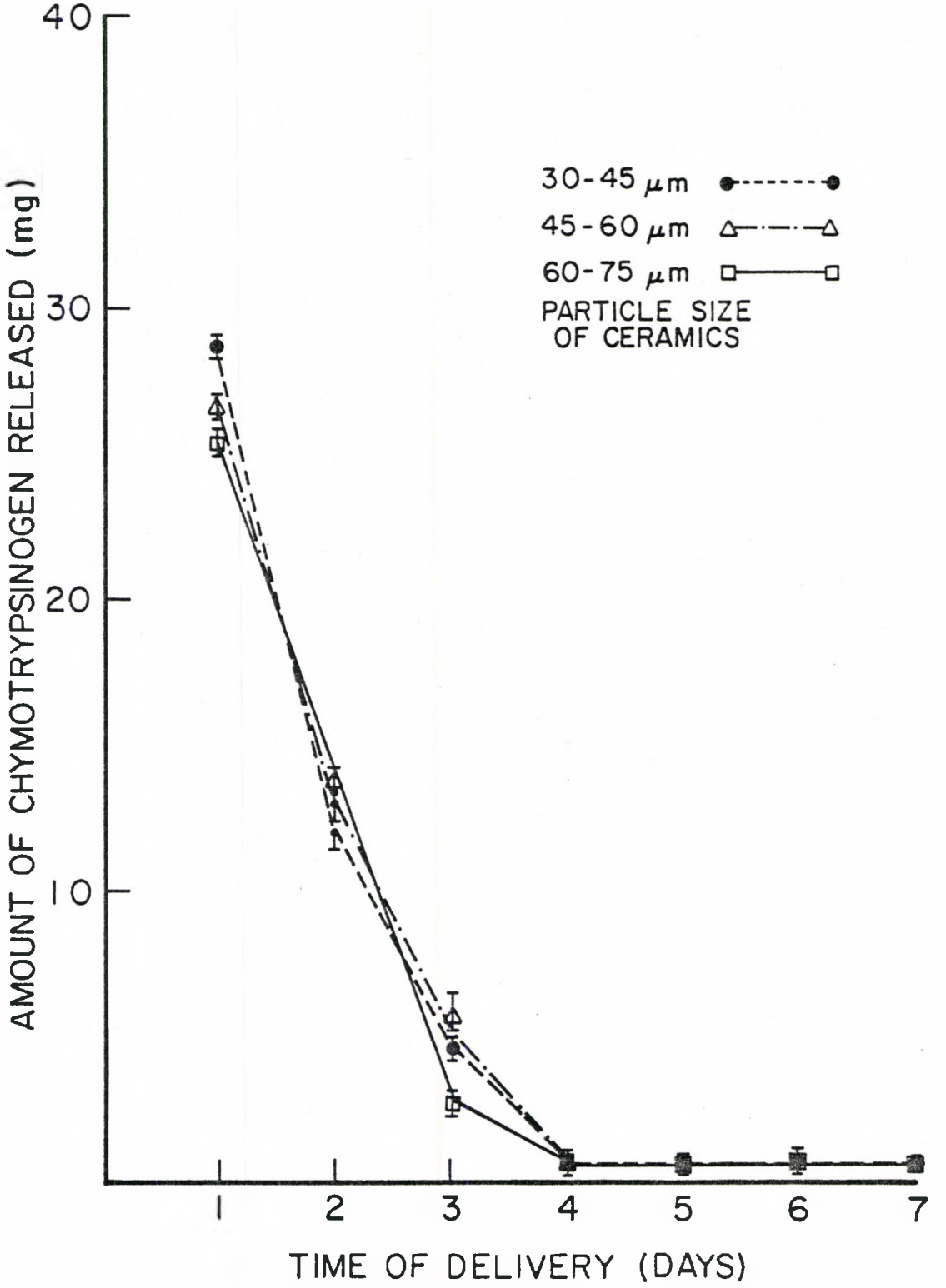
PARTICLE SIZE (μm)	AMOUNT OF CHYMOTRYPSINOGEN-A (mg)							
	DAY (1)	DAY (2)	DAY (3)	DAY (4)	DAY (5)	DAY (6)	DAY (7)	TOTAL
60-75	28.7 ± 0.2 AI	12.4 ± 0.3 BI	4.7 ± 0.2 CI	0.8 ± 0.1 DI	0.7 ± 0.1 EI	0.8 ± 0.1 DI	0.7 ± 0.1 EI	48.8 ± 0.3
45-60	26.8 ± 0.2 AII	13.4 ± 0.2 BII	5.5 ± 0.3 CII	0.7 ± 0.1 DII	0.7 ± 0.1 DI	0.7 ± 0.1 DII	0.7 ± 0.1 DI	48.5 ± 0.6
35-45	25.6 ± 0.2 AIII	13.5 ± 0.3 BII	2.6 ± 0.2 CIII	0.7 ± 0.2 DII	0.7 ± 0.1 DI	0.7 ± 0.2 DII	0.7 ± 0.1 DI	44.5 ± 0.4

*ALL DATA PRESENTED AS MEAN ± STANDARD DEVIATION

Mean ± S.D. values followed by different letters for each particle size (horizontally) are significant from each other at $P < 0.05$

Mean ± S.D. values followed by different Roman numerals for each day (vertically) are significant from each other at $P < 0.05$

Figure 5: Effect of particle size on the delivery profile of chymotrypsinogen-A by means of ALCAP ceramic capsules in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.


40
30
20
10
AMOUNT OF CHYMOTRYPSINOGEN RELEASED (mg)
30-45 μm
45-60 μm
60-75 μm
PARTICLE SIZE
OF CERAMICS
1
2
3
4
5
6
7
TIME OF DELIVERY (DAYS)

The delivery rate of chymotrypsinogen-A on the first day of the experiment was directly proportional to the starting particle size of the ceramics. On the second day there was no significant difference in the amount of chymotrypsinogen delivered by small and medium particle size ceramics. The amount of chymotrypsinogen-A delivered by the large particle size ceramics was significantly lower than the amount of chymotrypsinogen-A delivered by small and medium particle size ceramics. On the third day the lowest amount of chymotrypsinogen-A was delivered by the smallest particle size ceramics and the largest amount of chymotrypsinogen-A was delivered by the largest particle size ceramics. During the last four days although the statistical analysis showed differences in the amount of chymotrypsinogen-A delivered by the three different particle size ceramics it is apparent that the amounts of chymotrypsinogen-A delivered were too small and similar for logical interpretation.

Except for day to day variation the delivery profile of gamma globulin, bovine serum albumin, and chymotrypsinogen-A seems to follow a similar pattern.

Delivery of Insulin

The insulin data presented in table 4 and figure 6 shows that both ceramic particle size and passage of time significantly influenced the delivery rate of insulin. Differences in the amount of insulin delivered by ceramic replicates were not significant (appendix 4).

TABLE 4. MILLIGRAMS* OF INSULIN RELEASED DAILY (FOR SEVEN DAYS) IN PHOSPHATE BUFFERED SALINE (pH 7.4) EFFLUENT FROM ALCAP CERAMIC CAPSULES IN CONTINUOUS FLOW-THROUGH SYSTEM AT 37°C.

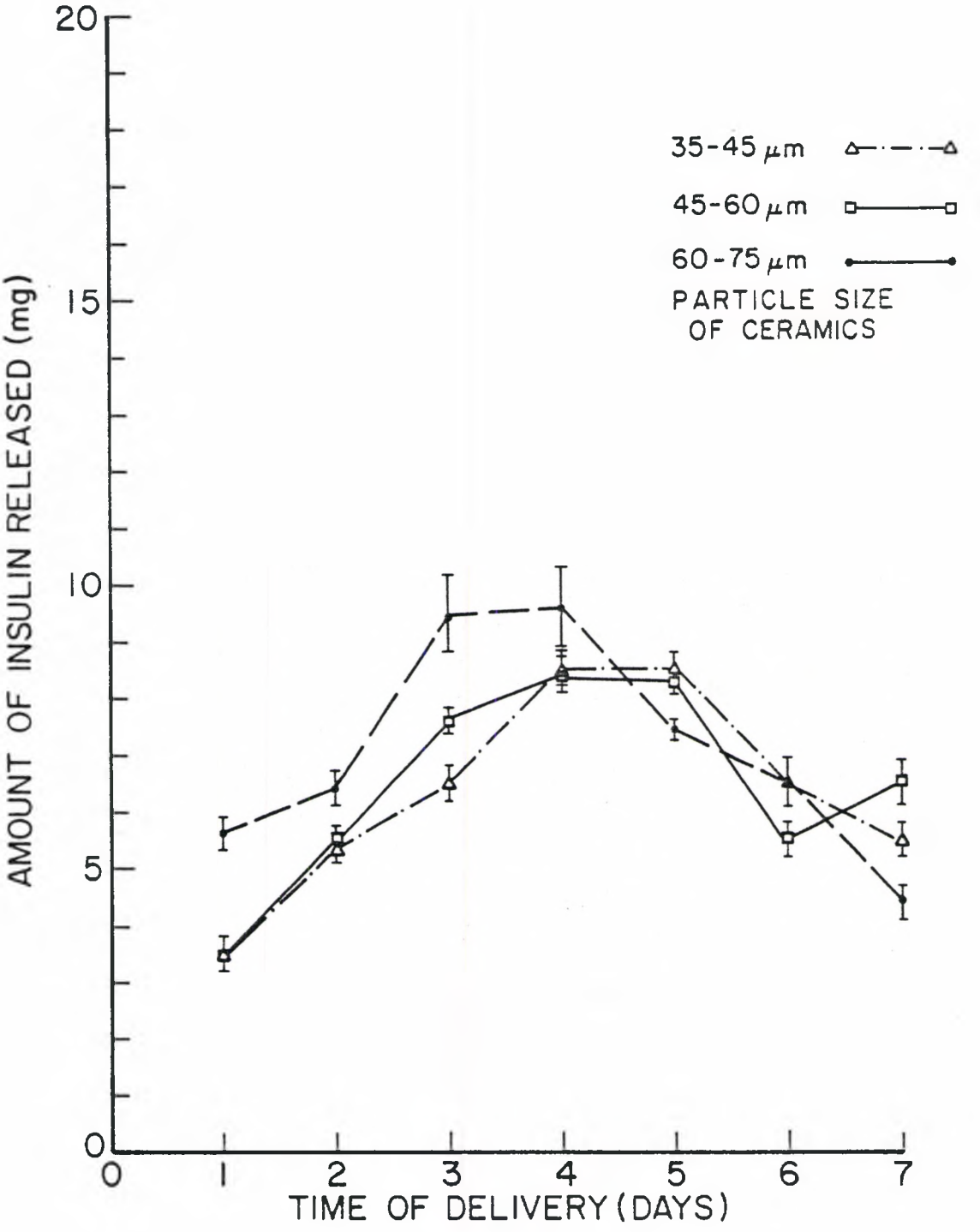
PARTICLE SIZE (μm)	AMOUNT OF INSULIN (mg)							
	DAY (1)	DAY (2)	DAY (3)	DAY (4)	DAY (5)	DAY (6)	DAY (7)	TOTAL
60-75	5.6 ± 0.3 AI	6.4 ± 0.3 BI	9.5 ± 0.7 CI	9.6 ± 0.7 CI	7.4 ± 0.2 DI	6.5 ± 0.3 BI	4.4 ± 0.3 EI	49.4 ± 0.4
45-60	3.5 ± 0.3 AII	5.5 ± 0.3 BII	7.6 ± 0.2 CII	8.4 ± 0.3 DII	8.3 ± 0.2 DII	5.5 ± 0.3 BII	6.5 ± 0.4 EII	45.3 ± 0.5
35-45	3.5 ± 0.3 AII	5.4 ± 0.3 BII	6.5 ± 0.3 CIII	8.5 ± 0.3 DII	8.5 ± 0.3 DII	6.5 ± 0.4 CI	5.5 ± 0.3 BIII	44.4 ± 0.7

*ALL DATA PRESENTED AS MEAN ± STANDARD DEVIATION

Mean ± S.D. values followed by different letters for each particle size (horizontally) are significant from each other at $P < 0.05$

Mean ± S.D. values followed by different Roman numerals for each day (vertically) are significant from each other at $P < 0.05$

Figure 6: Effect of particle size on the delivery profile of insulin by means of ALCAP ceramic capsules in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.


20
15
10
5
0
0
1
2
3
4
5
6
7
AMOUNT OF INSULIN RELEASED (mg)
TIME OF DELIVERY (DAYS)
35-45 μm
45-60 μm
60-75 μm
PARTICLE SIZE
OF CERAMICS

The amount of insulin released during the first two days by all the ceramics was significantly lower than the amount of insulin released during the next three days. The large particle size ceramics released the largest amount of insulin during the first four days of the trial. Differences in the amount of insulin released by small and medium particle size ceramics were not significant for the first two days and days four and five of the trial. A drop in the amount of insulin released took place after the fourth day in the case of largest particle size ceramics (60-75 um) and after the fifth day from small and medium particle size ceramics (35-45 and 45-60 um). The amount of insulin eluted from the largest particle size ceramic on days three and four were not significantly different and the amount of insulin eluted from the small and medium particle size ceramics were not significantly different. Up to the fourth day the delivery rate of insulin was directly proportional to the particle size. An inverse relationship between these two parameters seemed to take place during the last three days of the trial.

pH Data

Tables 5, 6, 7, and 8 show the pH of the phosphate buffered saline effluent on each day of the four trials conducted with different proteins in this investigation. After the initial drop in pH from 7.4 to 7.3 the range of pH changes in effluents collected from ceramic reservoirs containing gamma globulin, bovine serum albumin or chymotrypsinogen varied from 7.30 to 7.32 (tables 5-7). The range of pH change in effluents

collected from ceramic reservoirs containing insulin varied from 7.30 to 7.33 (table 8). Although the original pH shifted from 7.4 to 7.3 during the course of each trial, ceramic replicates, ceramic particle size, the nature of protein, and passage of time did not influence the pH of the effluent.

TABLE 5. Hydrogen ion concentration (pH) of Phosphate Buffered Saline (PBS) Collected Each Day (For Seven Days) From a Continuous Flow-Through System of PBS (Starting pH 7.4) Passing ALCAP Ceramic Capsules Containing Gamma Globulin at 37°C

PARTICLE SIZE (um)	DAY (1) pH	DAY (2) pH	DAY (3) pH	DAY (4) pH	DAY (5) pH	DAY (6) pH	DAY (7) pH
60-75	7.30 ± 0.01	7.31 ± 0.01	7.30 ± 0.01	7.32 ± 0.01	7.31 ± 0.01	7.30 ± 0.01	7.31 ± 0.01
45-60	7.31 ± 0.03	7.30 ± 0.01	7.31 ± 0.02	7.32 ± 0.01	7.32 ± 0.02	7.31 ± 0.01	7.30 ± 0.00
35-45	7.31 ± 0.02	7.31 ± 0.02	7.32 ± 0.00	7.30 ± 0.01	7.31 ± 0.01	7.31 ± 0.01	7.31 ± 0.01

* Data presented as mean ± standard deviation

TABLE 6. Hydrogen ion concentration (pH) of Phosphate Buffered Saline (PBS) Collected each day (For Seven Days) From a Continuous Flow-Through System of PBS (Starting pH 7.4) Passing ALCAP Ceramic Capsules Containing Bovine Serum Albumin at 37°C.

PARTICLE SIZE (um)	DAY (1)	DAY (2)	DAY (3)	DAY (4)	DAY (5)	DAY (6)	DAY (7)
	pH	pH	pH	pH	pH	pH	pH
60-75	7.31 ± 0.01	7.30 ± 0.01	7.30 ± 0.01	7.32 ± 0.02	7.31 ± 0.01	7.31 ± 0.01	7.31 ± 0.01
45-60	7.30 ± 0.00	7.31 ± 0.00	7.31 ± 0.01	7.30 ± 0.01	7.31 ± 0.01	7.32 ± 0.03	7.30 ± 0.02
35-45	7.31 ± 0.01	7.32 ± 0.01	7.30 ± 0.01	7.31 ± 0.01	7.31 ± 0.01	7.31 ± 0.01	7.31 ± 0.01

* Data presented as mean ± standard deviation

TABLE 7. Hydrogen ion concentration (pH) of Phosphate Buffered Saline (PBS) Collected Each Day (For Seven Days) From a Continuous Flow-Through System of PBS (Starting pH 7.4) Passing ALCAP Ceramic Capsules Containing Chymotrypsinogen-A at 37°C.

PARTICLE SIZE (um)	DAY (1) pH	DAY (2) pH	DAY (3) pH	DAY (4) pH	DAY (5) pH	DAY (6) pH	DAY (7) pH
60-75	7.31 ± 0.02	7.31 ± 0.01	7.30 ± 0.01	7.31 ± 0.01	7.30 ± 0.01	7.32 ± 0.01	7.31 ± 0.01
45-60	7.32 ± 0.01	7.31 ± 0.01	7.31 ± 0.00	7.30 ± 0.00	7.31 ± 0.02	7.30 ± 0.03	7.32 ± 0.01
35-45	7.31 ± 0.01	7.31 ± 0.01	7.31 ± 0.01	7.31 ± 0.01	7.32 ± 0.01	7.31 ± 0.01	7.31 ± 0.01

* Data presented as mean ± standard deviation

TABLE 8. Hydrogen ion concentration (pH) of Phosphate Buffered Saline (PBS) Collected Each Day (For Seven Days) From a Continuous Flow-Through System of PBS (Starting pH 7.4) Passing ALCAP Ceramic Capsules Containing insulin at 37°C.

PARTICLE SIZE (um)	DAY (1) pH	DAY (2) pH	DAY (3) pH	DAY (4) pH	DAY (5) pH	DAY (6) pH	DAY (7) pH
60-75	7.32 ± 0.01	7.30 ± 0.02	7.31 ± 0.01	7.30 ± 0.01	7.30 ± 0.01	7.31 ± 0.01	7.31 ± 0.00
45-60	7.32 ± 0.01	7.31 ± 0.01	7.30 ± 0.03	7.31 ± 0.02	7.31 ± 0.00	7.32 ± 0.03	7.30 ± 0.02
35-45	7.30 ± 0.01	7.31 ± 0.01	7.33 ± 0.03	7.32 ± 0.01	7.31 ± 0.01	7.33 ± 0.01	7.31 ± 0.01

* Data presented as mean ± standard deviation

DISCUSSION

In vitro data of Khot, et al. (1980) showed that insulin could be delivered by means of porous ceramics in a static system. Khot, et al. (1980) also suggested that by varying the particle size of the ceramic one can tailor the pore size and thus the delivery of substances of various molecular weights.

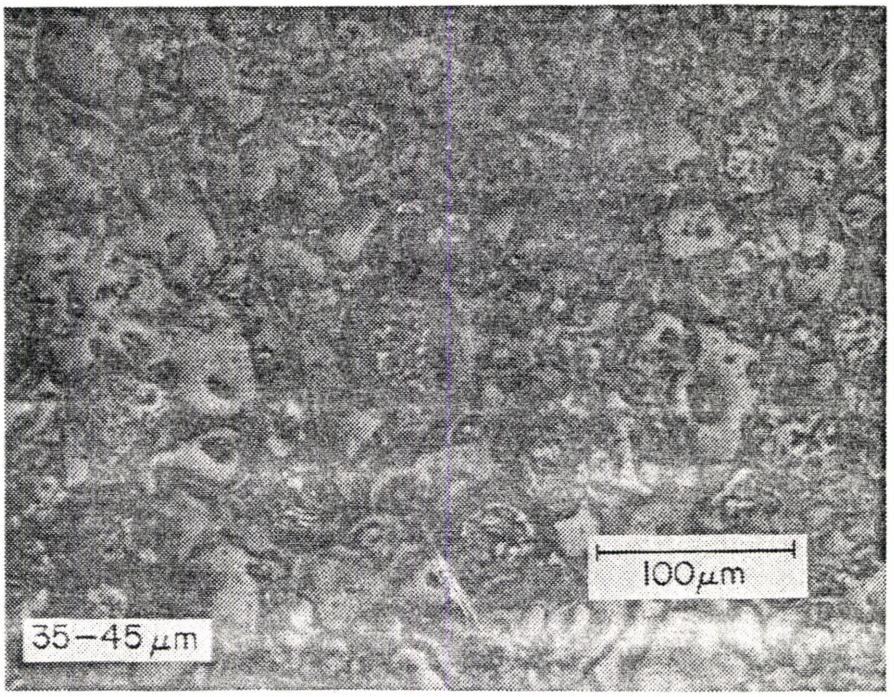
In vitro and in vivo studies conducted by Mattie, et al. (1980) and Bajpai, et al. (1976) have shown that ALCAP ceramics are resorbable, hence with passage of time the pore size of the ceramic should increase and compensate for the decrease in concentration of the substance in the reservoir by increasing the flow rate of the substance from the ceramic. Since ceramics are biocompatible and are porous in nature, it is logical to assume that they can deliver polypeptides or other substances of various molecular weights.

In my opinion there are three main forces responsible for delivering the polypeptides from the ceramic reservoir to the surrounding buffer medium: (1) concentration gradient between the ceramic cavity and the surrounding medium, (2) pressure developed within the ceramic cavity due to drawing in of the phosphate buffer saline by means of osmotic tension, and (3) the surface tension drag created by the phosphate

buffer saline flowing past the ceramic. On the other hand the opposing forces probably are: (1) absorptive nature of the ceramic particles, (2) trapping process of polypeptide molecules within the micropores between the ceramic particles, (3) adherence between polypeptides and ceramic particles due to interaction between charges of the proteins and ceramic components, (4) degree of viscosity displayed by each protein, and (5) probably the molecular size of the various proteins.

All of these postulates seem to be confirmed by the statistical analysis of the collected data (appendixes 1-4). Differences due to particle size and passage of time were evident. Differences due to nature of proteins were observed. No significant differences due to ceramic replicates were observed. Interactions between ceramic replicates and the different days were not significant except in some instances of protein delivery by the smallest particle size ceramics. The scanning electron micrographs of the cracked surface of a ceramic fabricated from the smallest particle size of 35-45 um (Fig. 7) shows the distribution of micro and macropores within the ceramic between sintered ceramic particles. The rate of gamma globulin delivery (Figs. 8-11) during the first two days was lower than the bovine serum albumin and chymotrypsinogen-A. Since the gamma globulin molecules are much larger and more viscous than the molecules of bovine serum albumin it appears that it takes almost two days for its molecules to saturate the macropores of the ceramics, after which the molecules are simply excluded due to differences in concentration gradient between

Figure 7: Scanning electron micrographs (250X) of a cracked surface of ALCAP ceramics fabricated from 35-45 um sized particles sintered at 1425°C for 24 hours showing the distribution of micropores and macropores within the ceramics. (Reproduced with a permission of D. R. Mattie, University of Dayton, Dayton, Ohio).


100μm
35–45 μm

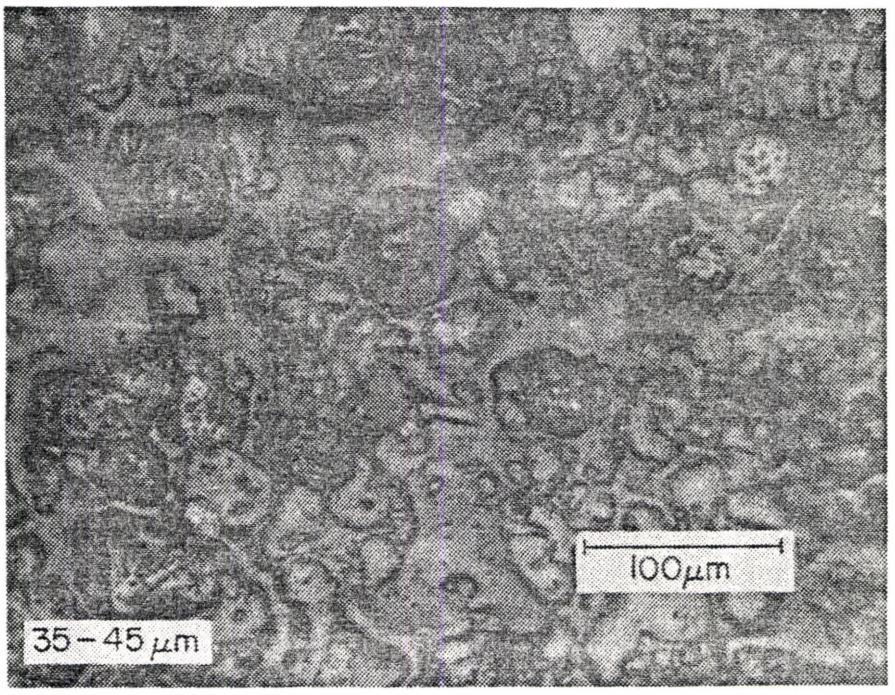

100μm
35–45 μm

Figure 8: Delivery profiles of gamma globulin, bovine serum albumin, chymotrypsinogen-A and insulin by ALCAP ceramic capsules fabricated from particle sizes ranging between 35 and 45 micrometers. The delivery took place in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.

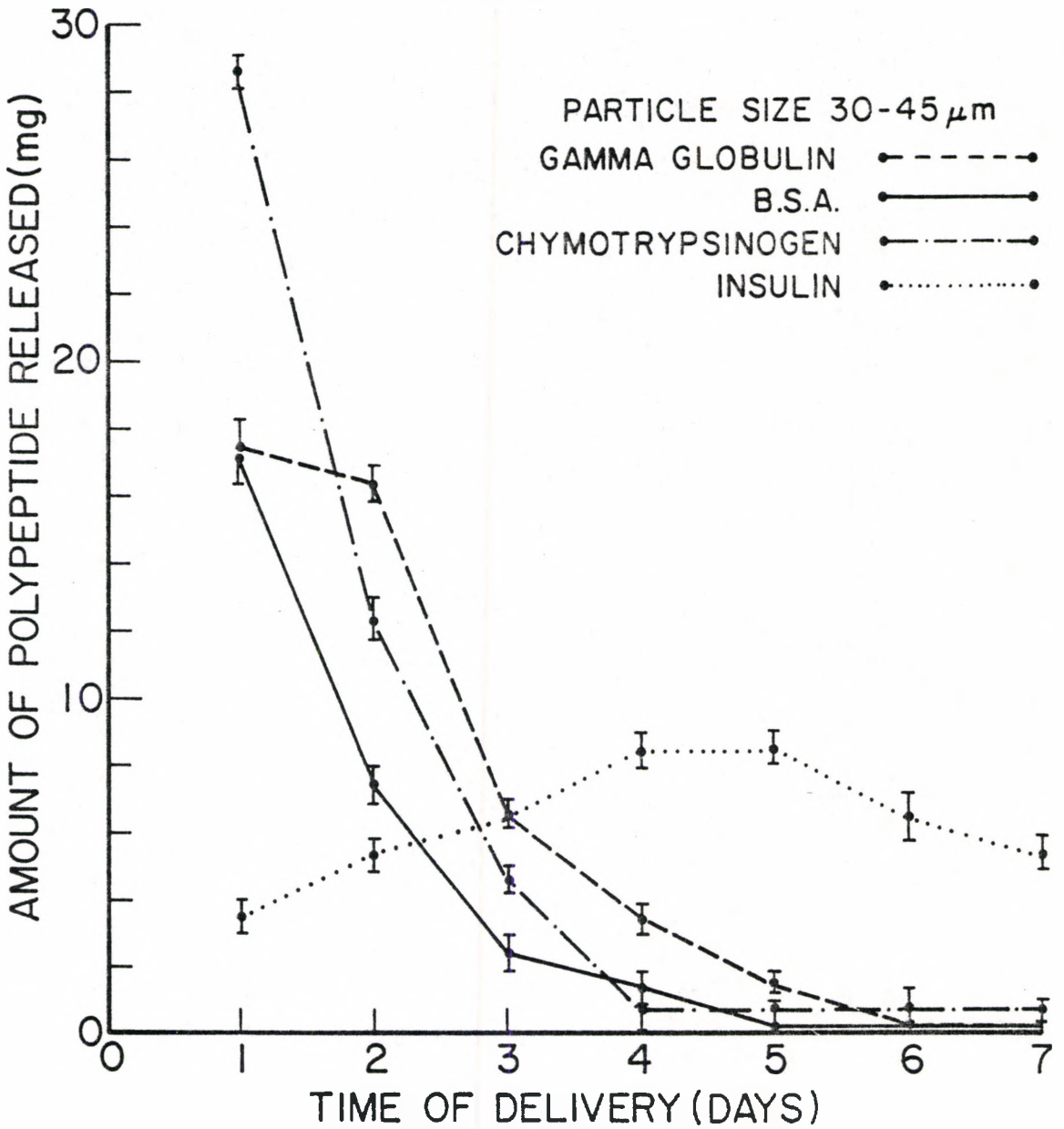

PARTICLE SIZE 30-45 μm
GAMMA GLOBULIN
B.S.A.
CHYMOTRYPSINOGEN
INSULIN
AMOUNT OF POLYPEPTIDE RELEASED(mg)
TIME OF DELIVERY (DAYS)
0
10
20
30
0
1
2
3
4
5
6
7

Figure 9: Delivery profiles of gamma globulin, bovine serum albumin, chymotrysinogen-A, and insulin by ALCAP ceramic capsules fabricated from particle sizes ranging between 45 and 60 micrometers. The delivery took place in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates $\pm$ the standard deviation.

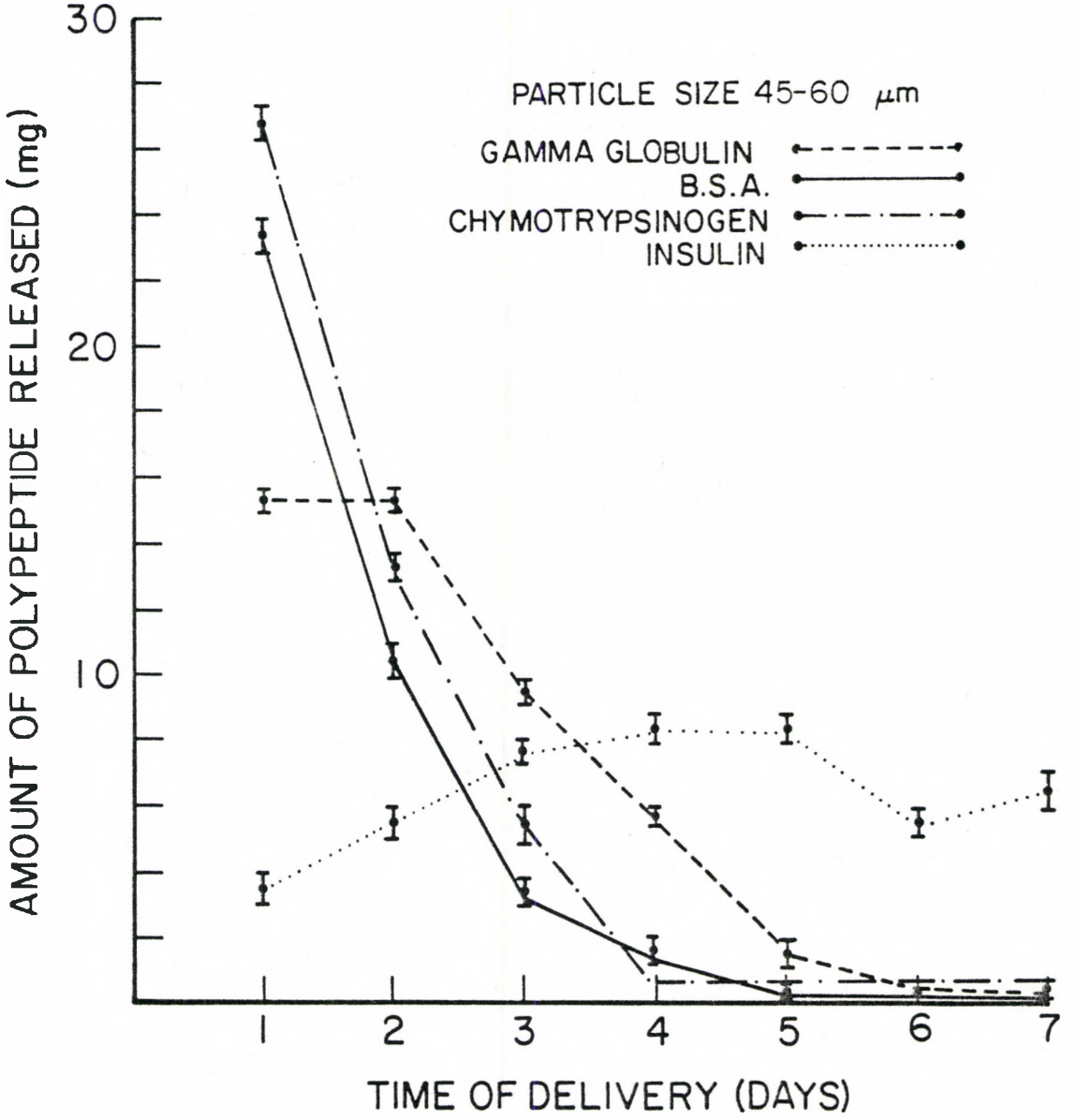

PARTICLE SIZE 45-60 μm
GAMMA GLOBULIN
B.S.A.
CHYMOTRYPSINOGEN
INSULIN
AMOUNT OF POLYPEPTIDE RELEASED (mg)
30
20
10
1
2
3
4
5
6
7
TIME OF DELIVERY (DAYS)

Figure 10: Delivery profiles of gamma globulin, bovine serum albumin, chymotrypsinogen-A, and insulin by ALCAP ceramic capsules fabricated from particle sizes ranging between 60 and 75 micrometers. The delivery took place in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.

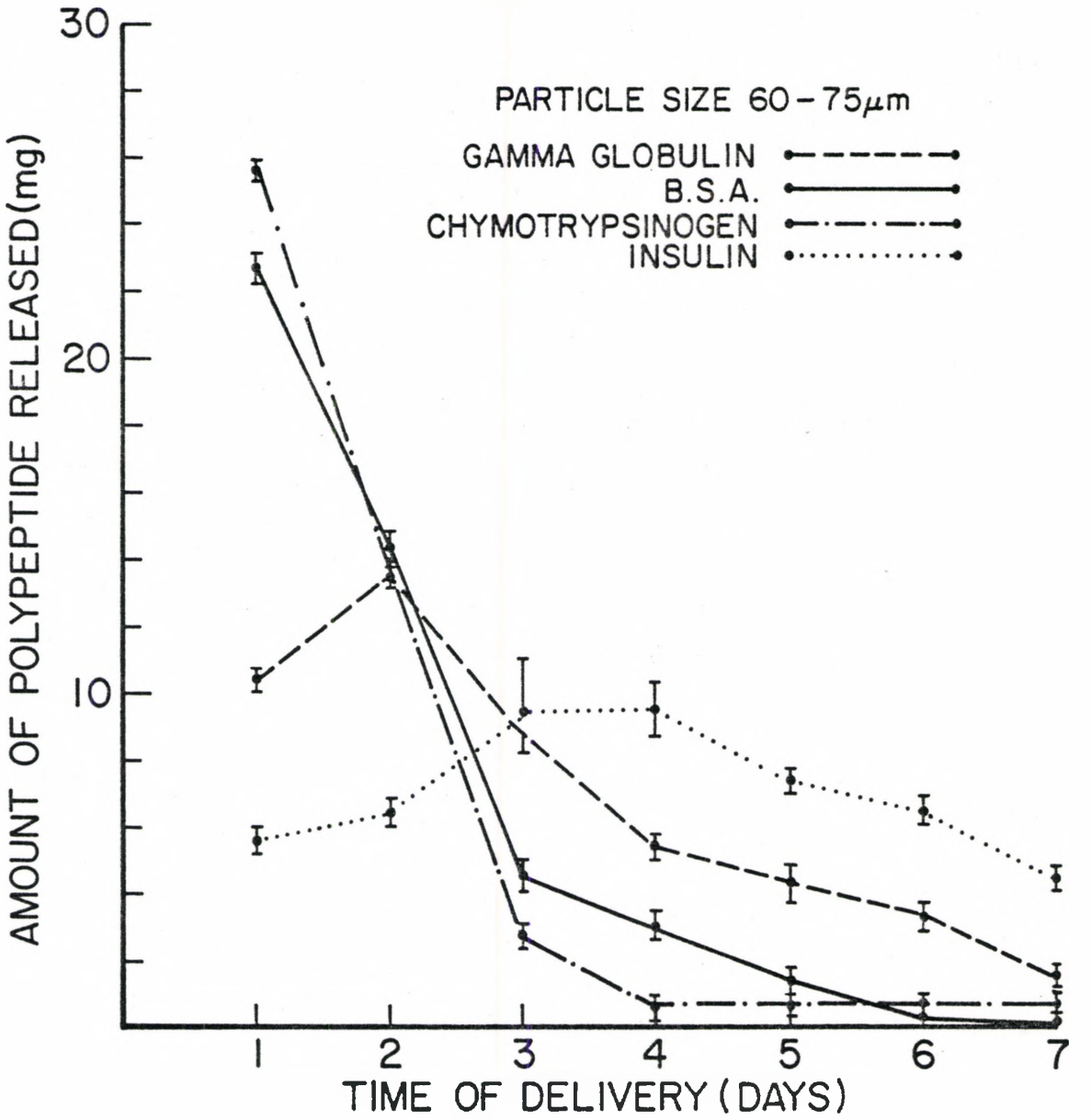
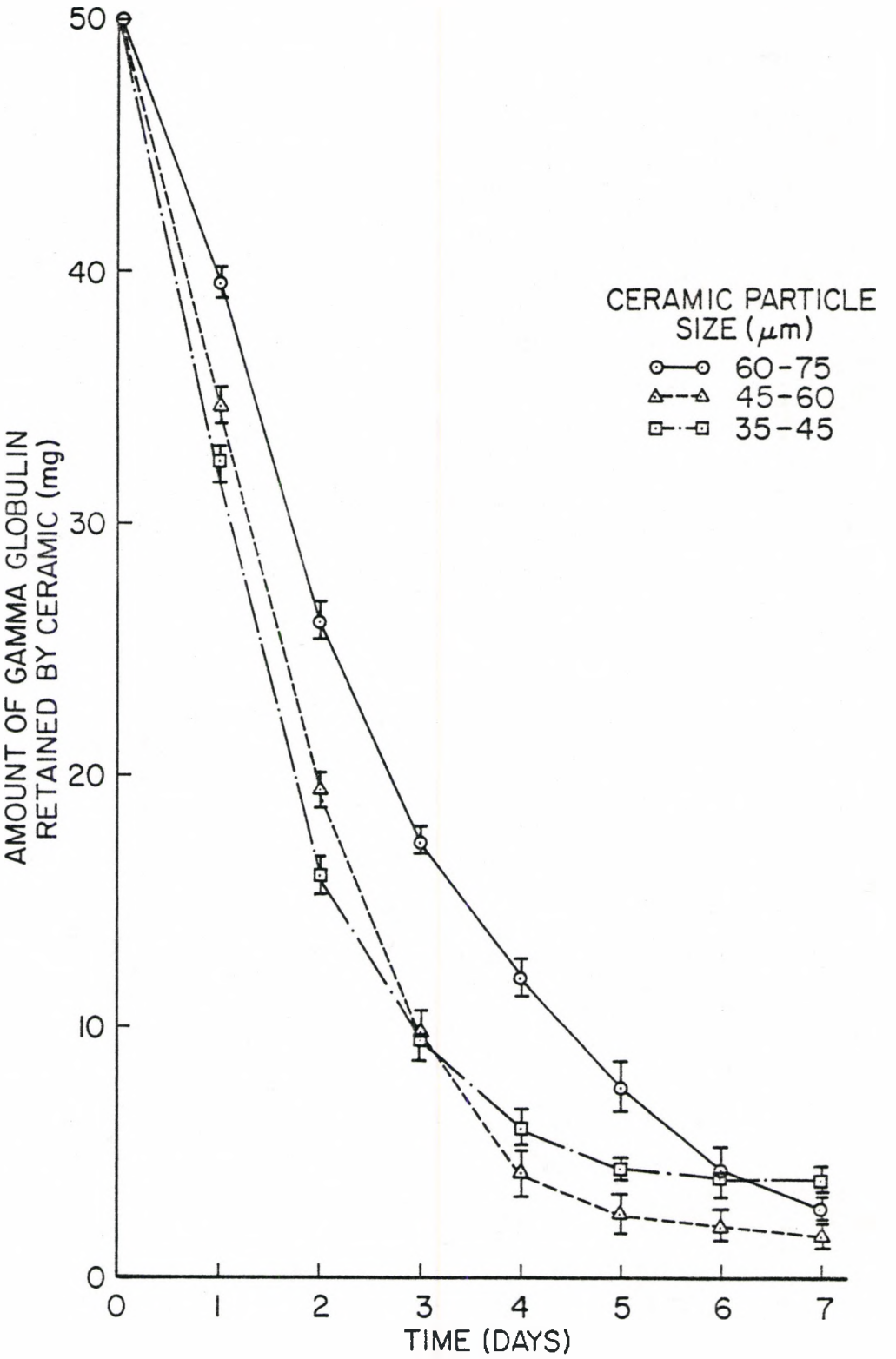

PARTICLE SIZE 60-75μm
GAMMA GLOBULIN
B.S.A.
CHYMOTRYPSINOGEN
INSULIN
AMOUNT OF POLYPEPTIDE RELEASED(mg)
30
20
10
1
2
3
4
5
6
7
TIME OF DELIVERY (DAYS)

Figure 11: Effect of particle size on the amount of gamma globulin retained by ALCAP ceramic capsules placed in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.


50
40
30
20
10
0
0
1
2
3
4
5
6
7
AMOUNT OF GAMMA GLOBULIN
RETAINED BY CERAMIC (mg)
TIME (DAYS)
CERAMIC PARTICLE
SIZE (μm)
60-75
45-60
35-45

the ceramic cavity and the surrounding buffer medium, and the surface tension drag created by the flowing of the buffer past the ceramic. The exclusion patterns of the bovine serum albumin (table 2, and figs. 8-10, and 12) and chymotrypsinogen-A (table 3, and figs. 8-10 and 13) were typical of chromatographic type of exclusion. However, after the first day of exclusion the amount of protein (gamma globulin, bovine serum albumin, and chymotrypsinogen-A) retained within the ceramic (figs. 11-13) started to govern the delivery profile of each polypeptide. The lower release of insulin during the first day of the trial could be due to the small molecular size of the insulin (figs. 8-10, and 14). Delay in the release of insulin molecule is probably due to the trapping of the molecules in the micropores of the ceramic material. This hypothesis seems to be confirmed by exponential phase of insulin release during the next two or three days of the experiment. The increase in insulin delivery in the following days is probably due to the saturation of the branched spaces between the ceramic particles by insulin molecules. This concept is supported by the leveling off of insulin delivery for a period of one day. The ensuring decrease in the rate of insulin delivery during the last three days of the experiment is probably due to the drop in concentration gradient and decrease in the amount retained in the ceramic reservoir (fig. 14). The pore

Figure 12: Effect of particle size on the amount of bovine serum albumin retained by ALCAP ceramic capsules placed in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.

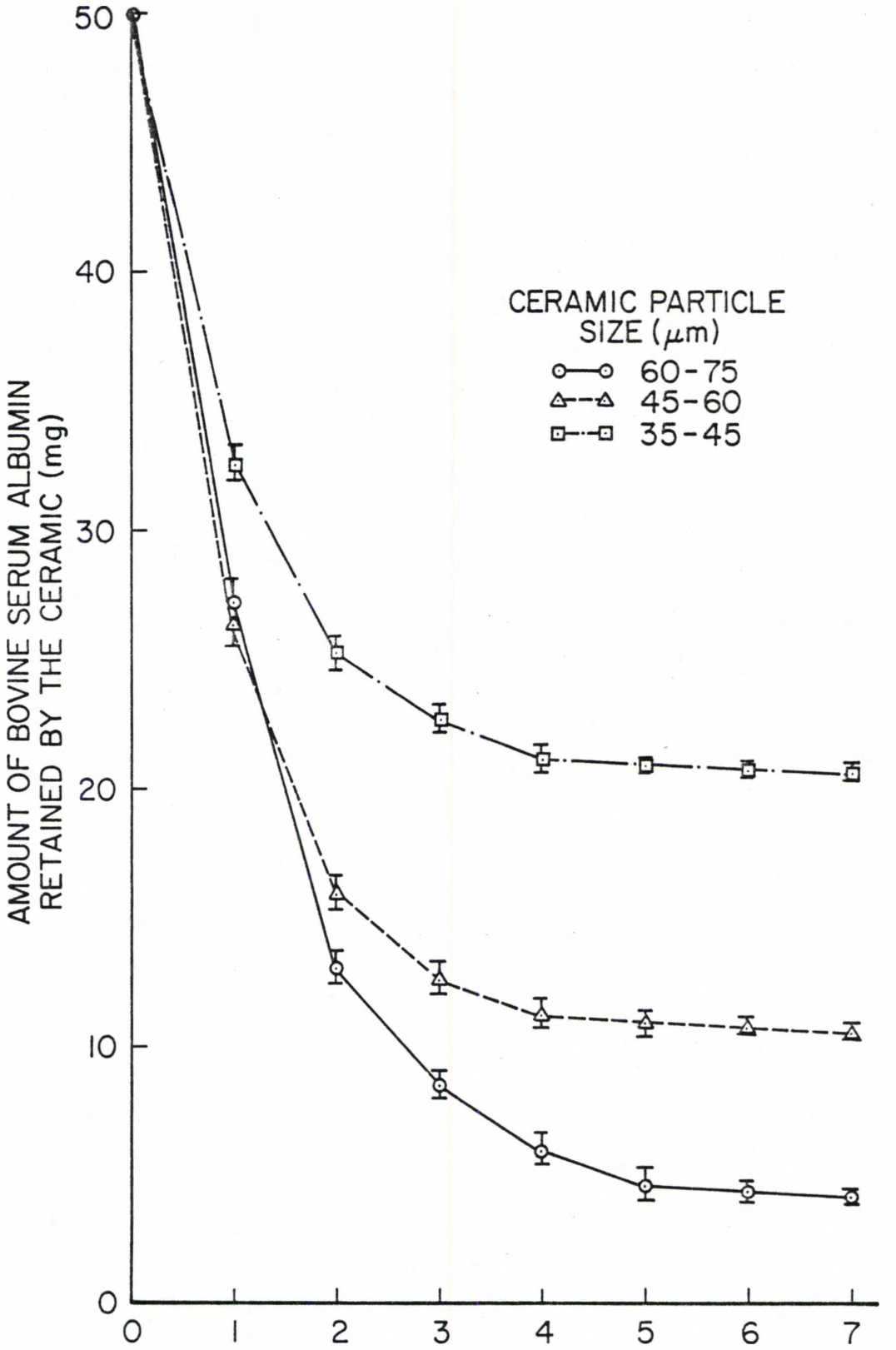

AMOUNT OF BOVINE SERUM ALBUMIN RETAINED BY THE CERAMIC (mg)
50
40
30
20
10
0
0
1
2
3
4
5
6
7
CERAMIC PARTICLE SIZE (μm)
60-75
45-60
35-45

Figure 13: Effect of particle size on the amount of chymotrypsinogen-A retained by ALCAP ceramic capsules placed in a continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the graphs represents a mean of eighteen replicates ± the standard deviation.

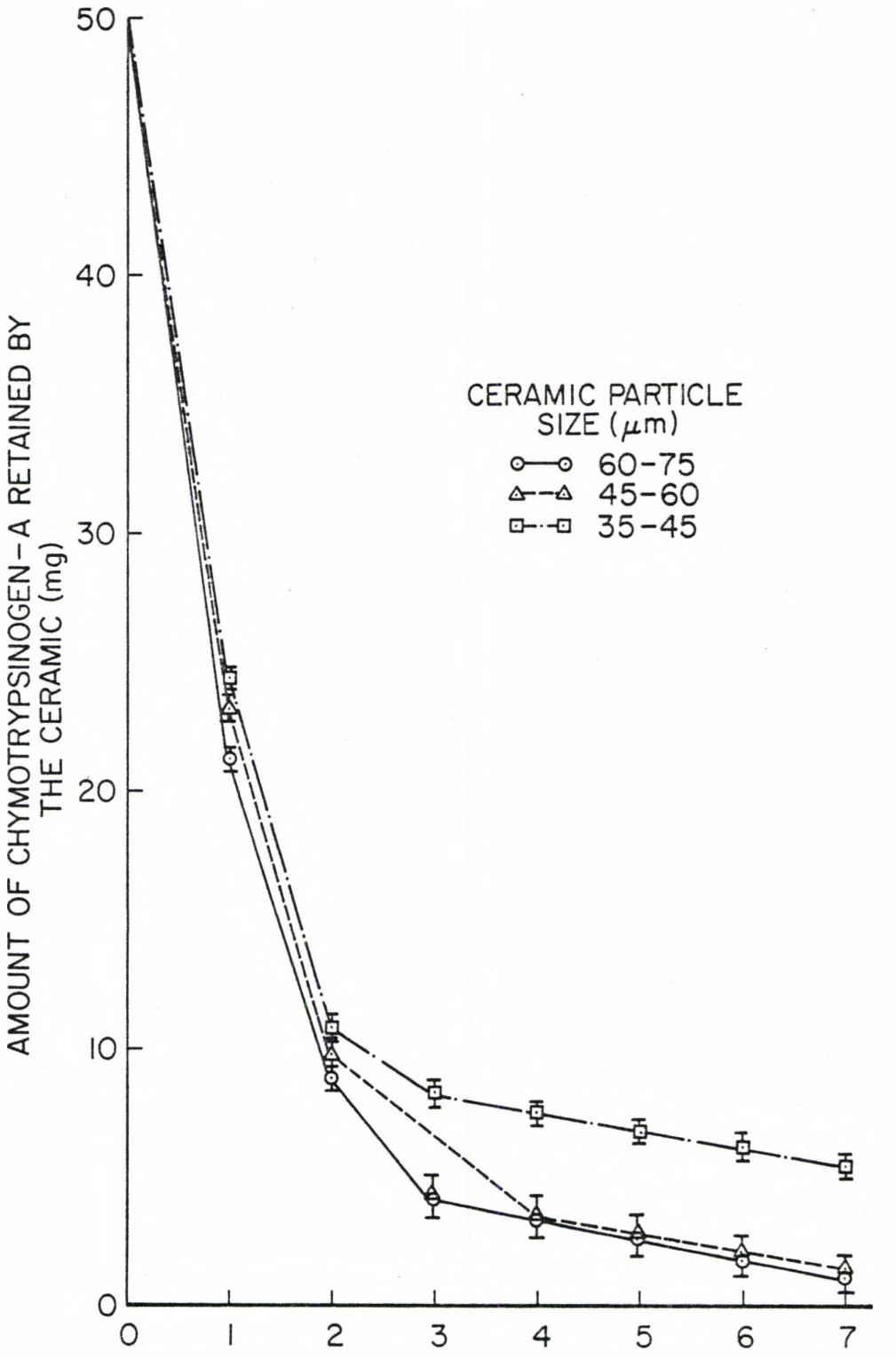
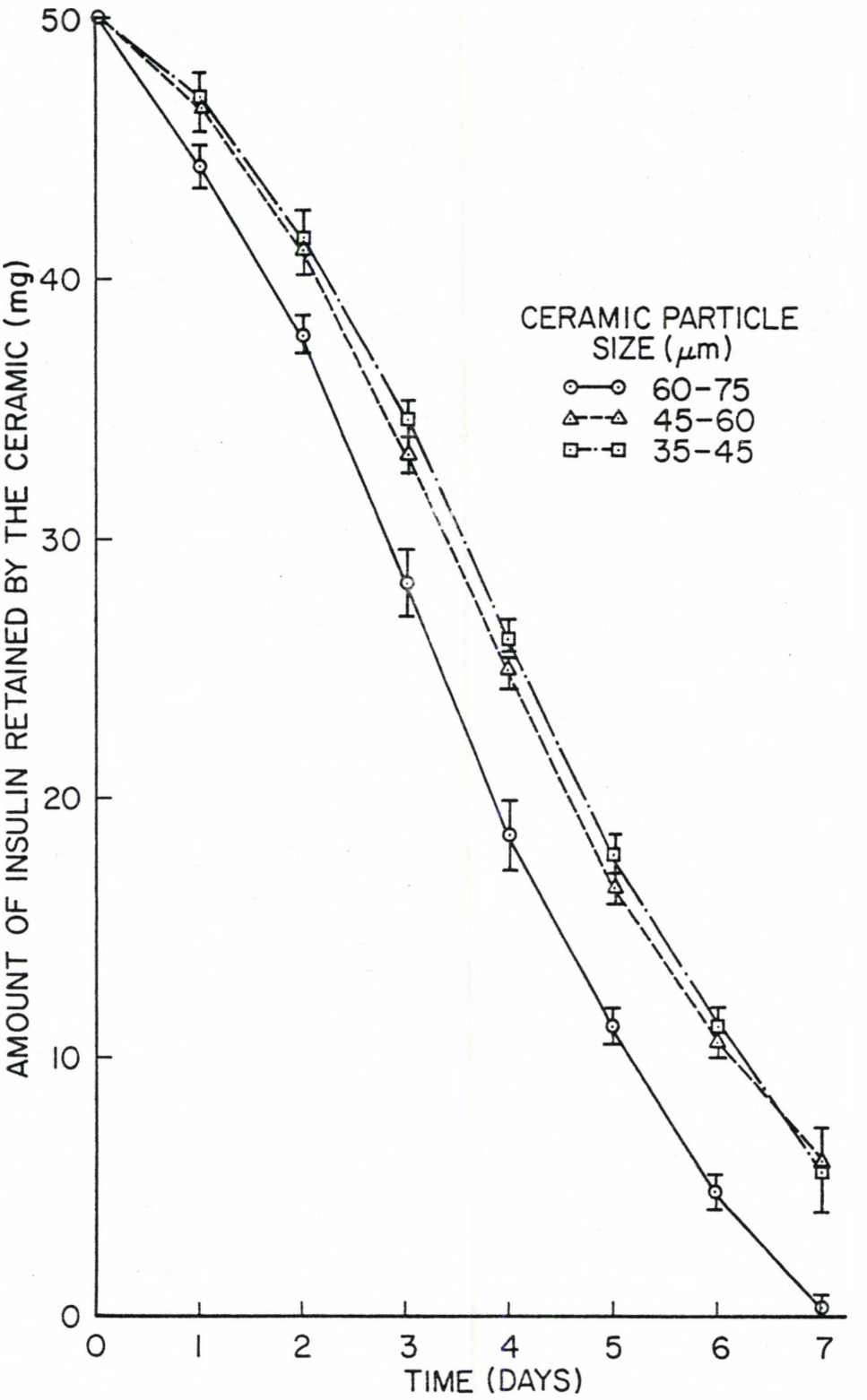
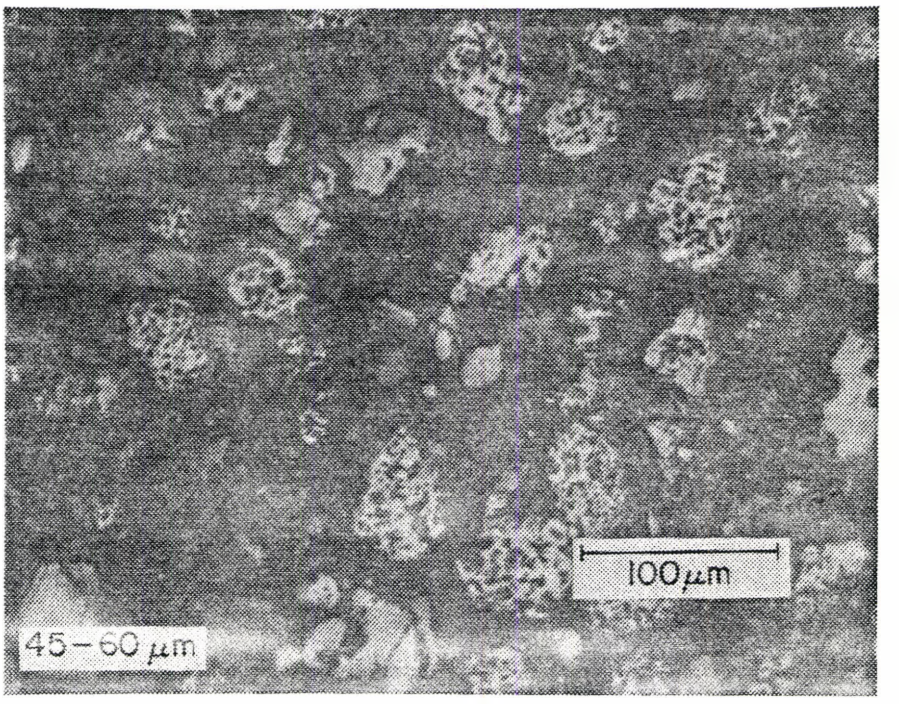

AMOUNT OF CHYMOTRYPSINOGEN-A RETAINED BY THE CERAMIC (mg)
50
40
30
20
10
0
0
1
2
3
4
5
6
7
CERAMIC PARTICLE SIZE (μm)
60-75
45-60
35-45

Figure 14: Effect of particle size on the amount of Insulin retained by ALCAP ceramic capsules placed in continuous flow-through system (0.2 ml/min) of phosphate buffered saline (pH 7.4) at 37°C for a total period of seven days. Each point on the represents a mean of eighteen replicates ± the standard deviation.


AMOUNT OF INSULIN RETAINED BY THE CERAMIC (mg)
TIME (DAYS)
CERAMIC PARTICLE SIZE (μm)
60-75
45-60
35-45
0
10
20
30
40
50
0
1
2
3
4
5
6
7

sizes of the ceramics fabricated from 35-45, 45-60, and 60-75 um size particles were 13.5 ± 2.2, 27.6 ± 3.1, and 32.3 ± um respectively (Figs. 7, 15 and 16). There is a direct relationship between the particle size and the pore size of the ceramic material. However it is also obvious that the number of macro pores per unit volume are inversely proportional to the particle size of the ceramic material. If the ceramics are behaving as chromatograph columns the direct relationship between the pore size and the particle size suggests that larger polypeptides should be excluded more rapidly by the smaller particle size ceramics and smaller peptides should be retained longer by the larger particle size ceramics. However, the largest amount of insulin was delivered by the largest particle size ceramics and this can be explained by the second relationship between the particle size and the number of pores per unit volume. It appears that the delay of the insulin delivery is affected not only by the trapping of the insulin molecules in the micropores of the ceramic material but also by the number of these pores per unit volume which means that the trapping process increases as the number of the micropores increase and macropores decrease per unit volume. The scanning electron micrographs of the medium and large particle size ceramics (Figs. 15 and 16) show less number of micropores, than that of the smallest particle size ceramics (Fig. 7). In short, delivery pattern of insulin (Table 4, and Figs. 8-10, and 14) suggests that the insulin molecules are either

Figure 15: Scanning electron micrographs (250X) of a cracked surface of ALCAP ceramics fabricated from 45-60 um sized particles sintered at 1425°C for 24 hours showing the distribution of the micropores and macropores within the ceramics. (Reproduced with a permission from D. R. Mattie, University of Dayton, Dayton, Ohio).


100 μm
45 – 60 μm

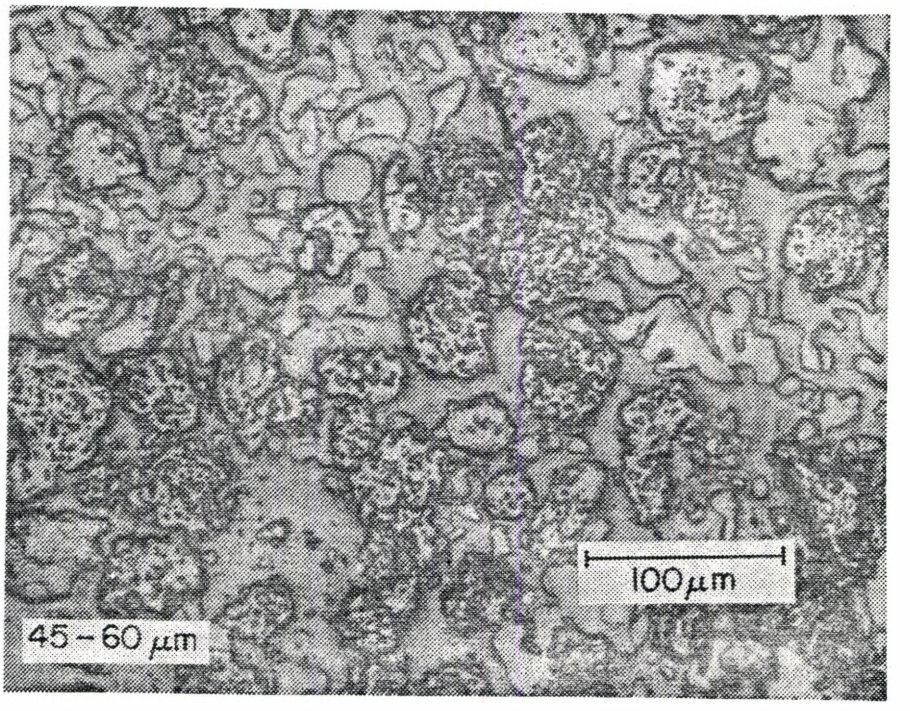
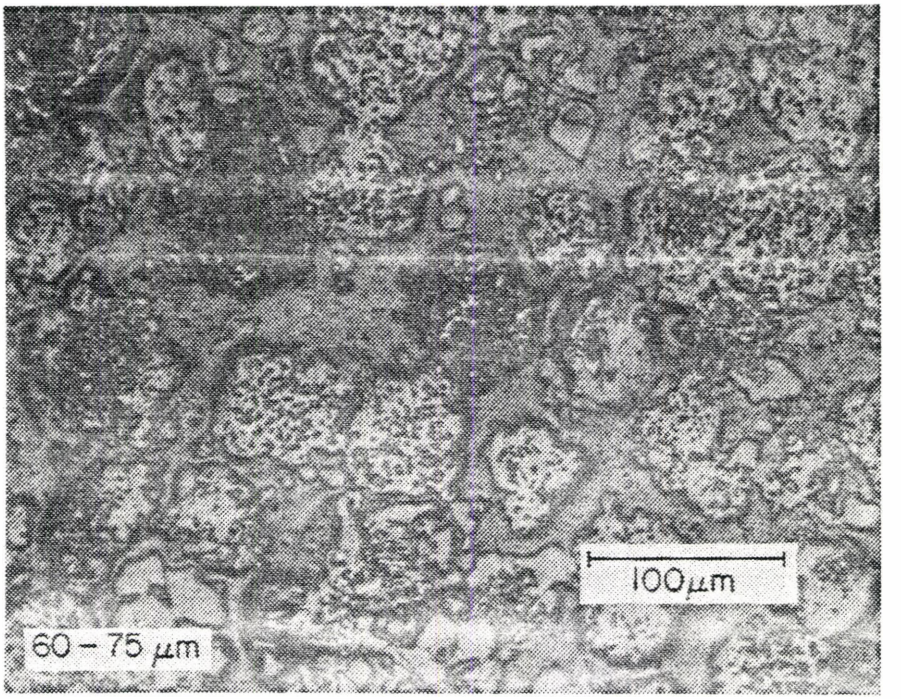
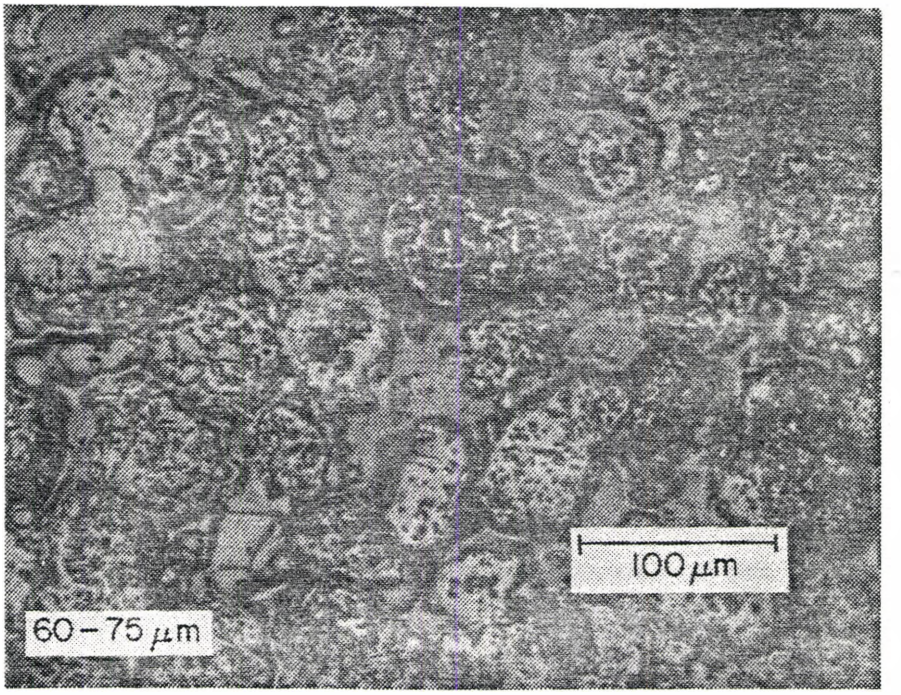

100 μm
45 – 60 μm

Figure 16: Scanning electron micrographs (250X) of a cracked surface of ALCAP ceramics fabricated from 60-75 um sized particles sintered at 1425°C for 24 hours showing the distribution of the micropores and macropores within the ceramics (reproduced with a permission from D. R. Mattie, University of Dayton, Dayton, Ohio).


100 μm
60 – 75 μm


100 μm
60 – 75 μm

trapped within the micropores or absorbed onto the ceramic particles for longer duration than the larger molecular weight polypeptides. This in turn allowed the insulin molecules to be delivered in a relatively sustained manner. Once again after the bulk of the polypeptide has been delivered during the first four days, the amount of insulin retained within the ceramic cavity (Fig. 14) starts to influence the delivery pattern. The delivery rate of bovine serum albumin (Table 2, and Fig. 12) increased by the decrease in the number of micropores. It is possible that the trapping process of the bovine serum albumin molecules within the micropores had the greatest effect on its delivery rate. The scanning electron micrograph of the largest particle size ceramics (Fig. 16), shows the least number of macropores. The rate of delivery of gamma globulin was the slowest by ceramics fabricated from the larger size particles (Table 1, and Fig. 11). This suggests that the molecules were excluded more slowly from ceramics containing less number of macropores. It is possible that the gamma globulin molecules were absorbed onto the larger particles for a longer time. Both viscosity and interaction of charges between the protein molecules and the ceramic particles could be responsible for this phenomenon.

The pH of the phosphate buffered saline effluent passing the ceramic capsules in the continuous flow-through system was not affected by any of the three variables: elution time; ceramic particle size; or the

molecular weight of the various polypeptides or proteins (Tables 5, 6, 7 and 8). However, the original pH shifted from 7.4 to 7.3 during the continuous flow studies. In the studies conducted by Khot, et al. (1980), and Mattie et al. (1981) with ceramics in static systems, it was obvious that the pH of the phosphate buffered saline or plasma (respectively) always shifted toward the alkali side. This change in pH was attributed to the rapid release of the hydroxyl ions from the ceramic into the effluent. Rapid replacement of the buffer medium in the continuous flow system probably prevented the accumulation of hydroxyl ions in this system. Since the pH of phosphate buffered saline with and without ceramics in the buffer changed from 7.4 to 7.3 it is likely that the carbon dioxide (CO_2) from the air coming through the 18G needle into the phosphate buffered saline solution was responsible for shifting the pH toward the acid side.

CONCLUSION

The data obtained from this investigation suggests that ALCAP ceramic reservoirs (capsules) can deliver polypeptides of various molecular weights. The ceramic reservoirs do not act as simple filtering devices but more like chromatographs and that the delivery of each peptide is governed by (1) the physical nature of the polypeptide, (2) the size of particles from which the ceramic was fabricated, and (3) the concentration gradient as determined by the amount of protein retained within the ceramic resevoir.

In short the ceramic reservoirs can be used as in-vivo depots for delivering active forms of polypeptides and proteins.

APPENDIX (1)

A

Analysis of variance tables for determination of significant differences (at $P < 0.05$) among particle sizes and ceramic replicates during the delivery of Gamma Globulin (mg) by ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (ph 7.4) at 37°C for a period of seven days.

Abbreviation Key

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean of Squares

Cer.Rep. = Ceramic capsule replicates

Part. Size = Ceramic particle size (Mm)

LSR = Least significant range.

K = The number of items over which the range is computed.

N = Number of data point used to determine the calculated value.

DAY (1)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	471.176	2	235.588	0.13×10^{-7}	1272.190*
Cer.Rep.	1.111	6	0.185	0.019	2.864 *
Error	2.910	45	0.065		

	Particle Size (u m)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	17.2 ± 0.2	15.3 ± 0.3	10.5 ± 0.4
2	17.7 ± 0.2	15.5 ± 0.3	10.3 ± 0.3
3	17.5 ± 0.3	15.2 ± 0.0	10.5 ± 0.2
MEAN	17.5 ± 0.3	15.3 ± 0.3	10.4 ± 0.3

* = Significant at P<0.05
NS = Not significant at P<0.05

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (2)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	84.342	2	42.171	0.216×10^{-5}	229.099 *
Cer.Rep.	1.104	6	0.184	0.067	2.140 NS
Error	3.870	45	0.086		

	Particle Size (um)		
Cer.Rep.	35-45	45-60	60-75
	(mg)	(mg)	(mg)
1	16.6 ± 0.3	15.7 ± 0.4	13.4 ± 0.3
2	16.6 ± 0.2	15.5 ± 0.3	13.3 ± 0.1
3	16.3 ± 0.2	15.4 ± 0.2	13.8 ± 0.2
MEAN	16.5 ± 0.3	15.5 ± 0.3	13.5 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (3)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	90.803	2	45.402	0.12×10^{-6}	605.359 *
Cer. Rep.	0.450	6	0.075	0.442	0.993 NS
Error	3.400	45	0.076		

	Particle Size (um)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	6.5 ± 0.3	9.4 ± 0.2	8.8 ± 0.2
2	6.3 ± 0.4	9.6 ± 0.3	8.7 ± 0.3
3	6.5 ± 0.2	9.5 ± 0.3	8.6 ± 0.2
MEAN	6.5 ± 0.3	9.5 ± 0.3	8.7 ± 0.2

* = Significant at $P < 0.05$
NS = Not significant at $P < 0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (4)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	43.858	2	21.929	0.11×10^{-5}	288.817 *
Cer. Rep.	0.456	6	0.076	0.334	1.187 NS
Error	2.897	45	0.064		

	Particle Size (um)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	3.7 ± 0.3	5.6 ± 0.2	5.4 ± 0.2
2	3.6 ± 0.1	5.5 ± 0.2	5.3 ± 0.3
3	3.5 ± 0.4	5.7 ± 0.3	5.6 ± 0.2
MEAN	3.5 ± 0.3	5.6 ± 0.2	5.4 ± 0.3

* = Significant at $P < 0.05$
NS = Not significant at $P < 0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (5)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	91.863	2	45.932	0.14×10^{-6}	574.152 *
Cer.Rep.	0.480	6	0.080	0.418	1.031 NS
Error	3.492	45	0.078		

	Particle Size (um)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	1.4 ± 0.2	1.7 ± 0.3	4.3 ± 0.3
2	1.6 ± 0.1	1.5 ± 0.3	4.5 ± 0.4
3	1.7 ± 0.1	1.5 ± 0.1	4.2 ± 0.4
MEAN	1.6 ± 0.2	1.6 ± 0.3	4.4 ± 0.4

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (6)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part. Size	106.045	2	53.022	0.993×10^{-7}	644.866 *
Cer.Rep.	0.493	6	0.082	0.236	1.399 NS
Error	2.645	45	0.059		

	Particle Size (um)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	0.5 ± 0.3	0.4 ± 0.2	3.4 ± 0.3
2	0.2 ± 0.0	0.4 ± 0.3	3.2 ± 0.3
3	0.2 ± 0.1	0.5 ± 0.3	3.4 ± 0.2
MEAN	0.3 ± 0.2	0.5 ± 0.3	3.3 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.3 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (7)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part. Size	19.563	2	9.782	0.27×10^{-6}	463.345 *
Cer.Rep.	0.127	6	0.021	0.684	0.657 NS
Error	1.445	45	0.032		

	Particle Size (um)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	0.2 ± 0.1	0.4 ± 0.3	1.4 ± 0.2
2	0.2 ± 0.2	0.5 ± 0.1	1.6 ± 0.2
3	0.2 ± 0.1	0.4 ± 0.2	1.5 ± 0.2
MEAN	0.2 ± 0.1	0.4 ± 0.2	1.5 ± 0.2

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.2 (mg)	0.3 (mg)
LSR		
Part.Size	0.1 (mg)	0.2 (mg)

N for the mean = 18

Appendix 1-B

Analysis of variance tables for determination of significant differences (at $P<0.05$) among: Days, Ceramic Replicates, and Interaction between Days and Ceramic Replicates during the Delivery of Gamma Globulin (mg) by ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C.

Particle Size 35-45 um

Factors	S.S.	D.F.	M.S.	P	F-ratio
Day	5974.810	6	995.802	0.397×10^{-19}	5963.93 *
Cer.Rep.	0.076	2	0.038	0.489	0.721 NS
Cer.Rep.x Days	2.004	12	0.167	0.724×10^{-3}	3.154 *
Error	5.558	105	0.053		

Particle Size 45-60 um

Factors	S.S.	D.F.	M.S.	P	F-ratio
Days	4799.32	6	799.887	0.399×10^{-21}	12844.8 *
Cer.Rep.	0.105	2	0.053	0.458	0.786 NS
Cer.Rep.x Days	0.747	12	0.062	0.517	0.933 NS
Error	7.008	105	0.067		

Particle Size 60-75 um

Factors	S.S.	D.F.	M.S.	P	F-ratio
Days	1967.78	6	327.964	0.21×10^{-17}	3076.2 *
Cer.Rep.	0.144	2	0.072	0.400	0.924 NS
Cer.Rep.X Days	1.279	12	0.107	0.193	1.368 NS
Error	8.185	105	0.078		

S.S. = Sum of squares
D.F. = Degrees of freedom
M.S. = Mean of squares

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

Appendix 1-C

Comparison Among Means by Student Newman Keuls Test on Amount of Gamma Globulin (mg) Delivered by the different particle sizes of ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C on each day for a period of seven days.

DAYS	MEANS OF AMOUNT DELIVERED		
(1)	10.4^{L}	15.3^{m}	17.5^{s}
(2)	13.5^{L}	15.3	16.5^{s}
(3)	6.5^{s}	8.7^{L}	9.5^{m}
(4)	3.5^{s}	5.4^{L}	9.7^{m}
(5)	1.6^{s}	1.6^{m}	4.4^{L}
(6)	0.3^{s}	0.5^{m}	3.3^{L}
(7)	0.2^{s}	0.4^{m}	1.5^{L}

s = Particle size 35-45 um
m = Particle size 45-60 um
L = Particle size 60-75 um

APPENDIX (2)

A

Analysis of variance tables for determination of significant differences (at $P<0.05$) among particle sizes and ceramic replicates during the delivery of bovine serum albumin (mg) by ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C for a period of seven days.

Abbreviation Key

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean of Squares

Cer. Rep. = Ceramic capsule replicates

Part. Size = Ceramic particle size (Mm)

LSR = Least significant range.

K = The number of items over which the range is computed.

N = Number of data point used to determine the calculated value.

DAY (1)

ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	395.536	2	197.768	0.24×10^{-8}	2234.27 *
Cer.Rep.	0.531	6	0.089	0.454	0.974 NS
Error	4.090	45	0.091		

Cer.Rep.	Particle Sizes (um) 35-45 (mg)	45-60 (mg)	60-75 (mg)
1	17.5 ± 0.3	23.6 ± 0.1	22.7 ± 0.3
2	17.3 ± 0.2	23.5 ± 0.3	22.6 ± 0.3
3	17.5 ± 0.3	23.5 ± 0.2	22.5 ± 0.3
Mean	17.4 ± 0.3	23.5 ± 0.3	22.6 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.4 (mg)	0.4 (mg)

LSR

Part.Size	0.2 (mg)	0.3 (mg)

N for mean = 18

DAY (2)
ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	438.922	2	219.461	0.598×10^{-8}	16.49.73 *
Cer.Rep.	0.798	6	0.133	0.903	0.518 NS
Error	3.102	45	0.069		

Cer.Rep.	Particles (um) 35-45 (mg)	45-60 (mg)	60-75 (mg)
1	7.5 ± 0.2	10.3 ± 0.3	14.6 ± 0.1
2	7.6 ± 0.3	10.4 ± 0.3	14.4 ± 0.2
3	7.3 ± 0.1	10.5 ± 0.3	14.3 ± 0.2
Mean	7.4 ± 0.3	10.5 ± 0.3	14.4 ± 0.2

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)

LSR

Part.Size	0.2 (mg)	0.2 (mg)

N for mean = 18

DAY (3)

ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	35.335	2	17.668	0.130 x 10	124.672*
Cer.Rep.	0.850	6	0.142	0.908	0.511 NS
Error	3.257	45	0.072		

Cer.Rep.	Particle Sizes (um)		
	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	2.4 ± 0.3	3.4 ± 0.1	4.7 ± 0.3
2	2.7 ± 0.2	3.4 ± 0.3	4.5 ± 0.2
3	2.5 ± 0.2	3.5 ± 0.2	4.3 ± 0.3
Mean	2.5 ± 0.3	3.4 ± 0.3	4.5 ± 0.3

* = Significant at $P < 0.05$
NS = Not significant at $P < 0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)

LSR

Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (4)

Factors	S.S.	D.F.	M.S.	P	F
Part.Size	15.225	2	7.613	0.61×10^{-3}	32.475*
Cer.Rep.	1.410	6	0.234	0.998	0.232 NS
Error	2.452	45	0.055		

Cer.Rep.	Particle Sizes (um) 35-45 (mg)	45-60 (mg)	60-75 (mg)
1	1.2 ± 0.3	1.6 ± 0.3	2.5 ± 0.3
2	1.7 ± 0.3	1.4 ± 0.2	2.5 ± 0.3
3	1.5 ± 0.2	1.2 ± 0.3	2.5 ± 0.3
Mean	1.5 ± 0.3	1.4 ± 0.3	2.5 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.3 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for mean = 18

DAY (5)

ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	16.883	2	8.441	0.137×10^{-4}	122.534 *
Cer.Rep.	0.413	6	0.069	0.031	2.555 *
Error	1.203	45	0.027	0.031	

Cer.Rep.	Particle Sizes (um) 35-45 (mg)	45-60 (mg)	60-75 (mg)
1	0.2 ± 0.0	0.2 ± 2	1.5 ± 0.3
2	0.2 ± 0.0	0.2 ± 1	1.5 ± 0.2
3	0.2 ± 0.1	0.2 ± 0	1.2 ± 0.3
Mean	0.2 ± 0.0	0.2 ± 0.1	1.4 ± 0.3

* - Significant at $P < 0.05$

K	2	3
Cer.Rep.	0.2 (mg)	0.2 (mg)
LSR		
Part.Size	0.1 (mg)	0.1 (mg)

N for the mean = 18

DAY (6)

Factors	S.S.	D.F.	M.S.	P	F
Part.Size	0.019	2	0.0096	0.196	2.167 NS
Cer.Rep.	0.027	6	0.0044	0.004	3.75 *
Error	0.053	45	0.0012		

Cer.Rep.	Particle Sizes (um)		
	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
2	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Mean	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.0 (mg)	0.0 (mg)

LSR

Part.Size	0.0 (mg)	0.0 (mg)

N for the mean = 18

DAY (7)

ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	0.058	2	0.029	0.237×10^{-2}	19.500 *
Cer.Rep.	0.009	6	0.0015	0.437	1 NS
Error	0.067	45	0.0015		

Cer.Rep.	Particle Sizes (um) 35-45 (mg)	45-60 (mg)	60-75 (mg)
1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
2	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
3	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Mean	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0

* = Significant at $P<0.05$
NS - Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.0 (mg)	0.0 (mg)

LSR

Part.Size	0.0 (mg)	0.0 (mg)

N for the mean = 18

Analysis of variance tables for determination of significant differences (at $P<0.05$) among: Days, Ceramic Replicates, and Interaction between Days and Ceramic Replicates during the Delivery of Gamma Globulin (mg) by ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C.

Particle Size 35-45 um

Factors	S.S.	D.F.	M.S.	P	F-ratio
Days	4384.79	6	730.799	0.256×10^{-19}	6417.6 *
Cer.Rep.	0.1535	2	0.0768	0.118	2.182 NS
Days x Cer.Rep.	1.3665	12	0.1139	0.548×10^{-3}	3.237 *
Error	3.6933	105	0.0352		

Particle Size 45-60 um

Factors	S.S.	D.F.	M.S.	P	F-ratio
Days	8191.11	6	1365.18	0.961×10^{-24}	35081.3 *
Cer.Rep.	0.01481	2	0.0074	0.8745	0.1347 NS
Day x Cer.Rep.	0.4670	12	0.0389	0.7408	0.7077 NS
Error	5.774	105	0.0550		

Particle Size 60-75 um

Factors	S.S.	D.F.	M.S.	P	F-ratio
Days	8089.73	6	1348.29	0.150×10^{-22}	22178.1 *
Cer.Rep.	0.661	2	0.330	0.305×10^{-2}	6.123 *
Days x Cer.Rep.	0.730	12	0.061	0.347	1.127 NS
Error	5.663	105	0.054		

S.S. = Sum of Squares
D.F. = Degrees of freedom
M.S. = Mean of Squares

* = Significant at $P<0.05$
NS = Insignificant at $P<0.05$

Comparison Among Means by Student-Newman Keuls Test on Amount of Bovine Serum Albumin (mg) Delivered by the Different Particle Sizes of ALCAP Ceramics in a Continuous Flow-Through System of Phosphate Buffered Saline (pH 7.4) at 37°C on each day for a period of seven days.

DAYS	MEANS OF AMOUNT DELIVERED		
1	17.4^{s}	22.6^{L}	23.5^{m}
2	7.4^{s}	10.5^{m}	14.4^{L}
3	2.5^{s}	3.4^{m}	4.5^{L}
4	1.4^{m}	1.5^{s}	2.5^{L}
5	0.2^{s}	0.2^{m}	1.4^{L}
6	0.2^{s}	0.2^{m}	0.2^{L}
7	0.1^{s}	0.1^{L}	0.2^{m}

s = Particle Size 35-45 um
m - Particle Size 45-60 um
L = Particle Size 60-75 um

APPENDIX (3)

A

Analysis of variance tables for determination of significant differences (at $P<0.05$) among particle sizes and ceramic replicates during the delivery of chymotrypsinogen (mg) by ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C for a period of seven days.

Abbreviation Key

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean of Squares

Cer.Rep. = Ceramic capsule replicates

Part.Size = Ceramic particle size (Mm)

LSR = Least significant range.

K = The number of items over which the range is computed.

N = Number of data point used to determine the calculated value.

DAY (1)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	91.813	2	45.907	0.18×10^{-6}	528.579 *
Cer.Rep.	0.521	6	0.869	0.103	1.891 NS
Error	2.067	45	0.459		

	Particle Size (um)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	25.7 ± 0.2	26.8 ± 0.2	28.9 ± 0.0
2	25.4 ± 0.2	26.7 ± 0.2	28.7 ± 0.3
3	25.6 ± 0.2	26.8 ± 0.2	28.7 ± 0.3
MEAN	25.6 ± 0.2	26.8 ± 0.2	28.7 ± 0.2

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.80 (mg)	1.00 (mg)
LSR		
Part.Size	0.50(mg)	0.60 (mg)

N for the mean = 18

DAY (2)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	14.450	2	7.220	0.219×10^{-5}	228.084 *
Cer.Rep.	0.190	6	0.032	0.853	0.434 NS
Error	3.290	45	0.073		

	Particle Size (um)		
Cer.Rep.	35-45	45-60	60-75
	(mg)	(mg)	(mg)
1	13.6 ± 0.3	13.5 ± 0.2	12.5 ± 0.3
2	13.6 ± 0.3	13.4 ± 0.3	12.3 ± 0.3
3	13.5 ± 0.3	13.5 ± 0.3	12.4 ± 0.3
MEAN	13.6 ± 0.3	13.4 ± 0.2	12.4 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.30 (mg)	0.40 (mg)
LSR		
Part.Size	0.20 (mg)	0.20 (mg)

N for the mean = 18

DAY (3)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	77.950	2	38.980	0.39×10^{-7}	880.62 *
Cer.Rep.	0.270	6	0.044	0.69	0.654 NS
Error	3.060	45	0.068		

Cer.Rep.	Particle Size (um) 35-45 (mg)	45-60 (mg)	60-75 (mg)
1	2.5 ± 0.3	5.5 ± 0.3	4.7 ± 0.2
2	2.7 ± 0.2	5.5 ± 0.3	4.6 ± 0.3
3	2.7 ± 0.2	5.5 ± 0.3	4.7 ± 0.1
MEAN	2.6 ± 0.2	5.5 ± 0.3	4.7 ± 0.2

* = Significant at $P<0.05$

NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.30 (mg)	0.40 (mg)
LSR		
Part.Size	0.20 (mg)	0.20 (mg)

N for the mean = 18

DAY (4)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	0.270	2	0.136	0.0065	13.070 *
Cer.Rep.	0.062	6	0.010	0.592	0.778 NS
Error	0.600	45	0.013		

	Particle Size (um)		
Cer.Rep.	35-45	45-60	60-75
	(mg)	(mg)	(mg)
1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
2	0.7 ± 0.0	0.7 ± 0.0	0.9 ± 0.1
3	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
MEAN	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1

* = Significant at $P < 0.05$
NS = Not significant at $P < 0.05$

K	2	3
Cer.Rep.	0.10 (mg)	0.20 (mg)

LSR

Part.Size	0.06 (mg)	0.09 (mg)

N for the mean = 18

DAY (5)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	0.040	2	0.020	0.064	4.500 NS
Cer.Rep.	0.027	6	0.004	0.843	0.448 NS
Error	0.447	45	0.010		

	Particle Size (um)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.0
2	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
3	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.1
MEAN	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.10 (mg)	0.20 (mg)

LSR

Part.Size	0.06 (mg)	0.08 (mg)

N for the mean = 18

DAY (6)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	0.085	2	0.042	0.026	7.125 *
Cer.Rep.	0.036	6	0.006	0.919	0.328 NS
Error	0.813	45	0.018		

	Particle Size (um)		
Cer.Rep.	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.1
2	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.1
3	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.1
MEAN	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.1

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.20 (mg)	0.20 (mg)

LSR

Part.Size	0.09 (mg)	0.1 (mg)

N for the mean = 18

DAY (7)

Factors	S.S.	D.F.	M.S.	P	F-ratio
Part.Size	0.028	2	0.014	0.086	3.800 NS
Cer.Rep.	0.022	6	0.004	0.881	0.391 NS
Error	0.427	45	0.010		

	Particle Size (um)		
Cer.Rep.	35-45	45-60	60-75
	(mg)	(mg)	(mg)
1	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
3	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
MEAN	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.10 (mg)	0.20 (mg)
LSR		
Part.Size	0.06 (mg)	0.08 (mg)

N for the mean = 18

Analysis of variance tables for determination of significant differences (at $P<0.05$) among: Days, Ceramic Replicates, and Interaction between Days and Ceramic Replicates during the Delivery of Gamma Globulin (mg) by ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C.

Particle Size 35-45 um

Factors	S.S.	D.F.	M.S.	P.	F-ratio
Days	10136.000	6	1689.330	0.73×10^{-24}	36742.6 *
Cer. Rep.	0.005	2	0.003	0.932	0.071 NS
Cer. Rep. x Days	0.552	12	0.046	0.217	1.321 NS
Error	3.653	105	0.35		

Particle Size 45-60 um

Factors	S.S.	D.F.	M.S.	P.	F-ratio
Days	10680.500	6	1780.090	0.348×10^{-27}	131424 *
Cer. Rep.	0.012	2	0.006	0.850	0.164 NS
Cer. Rep. x Days	0.163	12	0.014	0.970	0.373 NS
Error	3.815	105	0.036		

Particle Size 60-75 um

Factors	S.S.	D.F.	M.S.	P.	F-ratio
Days	11918.200	6	1986.400	0.14×10^{-25}	70902.5 *
Cer. Rep.	0.101	2	0.050	0.192	1.676 NS
Cer. Rep. x Days	0.336	12	0.028	0.516	0.934 NS
Error	3.148	105	0.030		

S.S. = Sum of squares
D.F. = Degrees of freedom
M.S. = Mean of squares

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

Appendix 3-C

Comparison among Means by Student-Newman Keuls Test on Amount of Chymotrypsinogen (mg) Delivered by the Different Particle size of ALCAP ceramics in continuous Flow-Through System of Phosphate Buffered Saline (pH 7.4) at 37°C on each day for a period of seven days.

DAYS	MEANS OF AMOUNT DELIVERED		
1	25.6^{s}	26.8^{m}	28.7^{L}
2	12.4^{L}	13.4^{m}	13.5^{s}
3	2.6^{s}	4.7^{L}	5.5^{m}
4	0.7^{s}	0.7^{m}	0.8^{L}
5	0.7^{s}	0.7^{m}	0.7^{L}
6	0.7^{s}	0.7^{m}	0.8^{L}
7	0.7^{s}	0.7^{m}	0.7^{L}

s = Particle size 35-45 um
m = Particle size 45-60 um
L = Particle size 60-75 um

APPENDIX (4)

A

Analysis of variance tables for determination of significant differences (at $P<0.05$) among particle sizes and ceramic replicates during the delivery course of insulin (mg) by ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C for a period of seven days.

Abbreviation Key

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean of Squares

Cer. Rep. = Ceramic capsule replicates

Part. Size = Ceramic particle size (Mm)

LSR = Least significant range.

K = The number of items over which the range is computed.

N = Number of data point used to determine the calculated value.

DAY (1)

ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	57.231	2	28.625	0.255×10^{-5}	216.724 *
Cer.Rep.	0.792	6	0.132	0.127	1.770 NS
Error	3.357	45	0.075		

Cer.Rep.	Particle Sizes (um) 35-45 (mg)	45-60 (mg)	60-75 (mg)
1	3.5 ± 0.3	3.3 ± 0.2	5.7 ± 0.3
2	3.6 ± 0.2	3.5 ± 0.3	5.7 ± 0.1
3	3.3 ± 0.4	3.7 ± 0.3	5.6 ± 0.4
Mean	3.5 ± 0.3	3.5 ± 0.3	5.6 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)

LSR

Part. Size	0.2 (mg)	0.2 (mg)

N for the Mean = 18

DAY (2)

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	11.383	2	5.691	0.508×10^{-4}	78.002 *
Cer.Rep.	0.438	6	0.073	0.394	1.071 NS
Error	3.065	45	0.068		

Cer.Rep.	Particle Sizes (um)		
	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	5.5 ± 0.3	5.5 ± 0.3	6.3 ± 0.2
2	5.3 ± 0.3	5.5 ± 0.3	6.5 ± 0.3
3	5.5 ± 0.0	5.6 ± 0.2	6.5 ± 0.3
Mean	5.4 ± 0.3	5.5 ± 0.3	6.4 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for the Mean = 18

DAY (3)

ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	85.788	2	42.894	0.574×10^{-6}	358.003 *
Cer.Rep.	0.719	6	0.120	0.058	2.223 NS
Error	2.425	45	0.054		

Cer.Rep.	Particle Sizes (um)		
	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	6.5 ± 0.4	7.6 ± 0.3	9.6 ± 0.1
2	6.6 ± 0.1	7.5 ± 0.1	9.3 ± 0.2
3	6.4 ± 0.2	7.6 ± 0.3	9.7 ± 0.3
Mean	6.5 ± 0.3	7.6 ± 0.3	9.5 ± 0.7

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.3 (mg)

LSR

Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (4)

Factors	S.S.	D.F.	M.S.	P.	F
Part. Size	15.303	2	7.651	0.252×10^{-3}	*
Cer. Rep.	1.031	6	0.172	0.030	*
Error	2.972	45	0.066		

Cer. Rep.	Particle Sizes (um)		
	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	8.4 ± 0.3	8.2 ± 0.3	9.3 ± 0.1
2	8.5 ± 0.3	8.4 ± 0.2	9.6 ± 0.4
3	8.5 ± 0.2	8.6 ± 0.2	9.8 ± 0.2
Mean	8.5 ± 0.3	8.4 ± 0.3	9.6 ± 0.7

* = Significant at $P<0.05$
NS - Not significant at $P<0.05$

K	2	3
Cer. Rep.	0.3 (mg)	0.4 (mg)
LSR		
Part. Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (5)

ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part. Size	11.445	2	5.722	0.258×10^{-3}	44.144 *
Cer. Rep.	0.778	6	0.130	0.054	2.268 NS
Error	2.572	45	0.057		

Cer. Rep.	Particle Sizes (um)		
	35-45	45-60	60-75
	(mg)	(mg)	(mg)
1	8.2 ± 0.2	8.2 ± 0.2	7.5 ± 0.1
3	8.5 ± 0.3	8.3 ± 0.1	7.3 ± 0.3
3	8.7 ± 0.3	8.3 ± 0.3	7.4 ± 0.3
Mean	8.5 ± 0.3	8.3 ± 0.2	7.4 ± 0.2

* = Significant at $P < 0.05$
NS - Not significant at $P < 0.05$

K	2	3
Cer. Rep.	0.3 (mg)	0.3 (mg)

LSR

Part. Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (6)

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	11.967	2	5.984	0.221×10^{-2}	20.032 *
Cer.Rep.	1.792	6	0.299	0.237×10^{-2}	4.082 *
Error	3.293	45	0.073		

Cer.Rep.	Particle Sizes (um)		
	35-45 (mg)	45-60 (mg)	60-75 (mg)
1	6.7 ± 0.3	5.6 ± 0.3	6.5 ± 0.1
2	6.6 ± 0.3	5.7 ± 0.3	6.7 ± 0.3
3	6.1 ± 0.1	5.3 ± 0.3	6.4 ± 0.3
Mean	6.5 ± 0.4	5.5 ± 0.3	6.5 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.3 (mg)	0.4 (mg)
LSR		
Part.Size	0.2 (mg)	0.2 (mg)

N for the mean = 18

DAY (7)

ANOVA

Factors	S.S.	D.F.	M.S.	P.	F
Part.Size	14.303	2	7.151	0.879×10^{-4}	64.469 *
Cer.Rep.	0.666	6	0.111	0.311	1.225 NS
Error	4.073	45	0.091		

Cer.Rep.	Particle Sizes (um) 35-45 (mg)	45-60 (mg)	60-75 (mg)
1	5.6 ± 0.4	5.4 ± 0.2	4.5 ± 0.1
2	5.3 ± 0.3	5.4 ± 0.3	4.5 ± 0.4
3	5.7 ± 0.2	5.5 ± 0.3	4.2 ± 0.3
Mean	5.5 ± 0.3	6.5 ± 0.4	4.4 ± 0.3

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

K	2	3
Cer.Rep.	0.4 (mg)	0.4 (mg)

LSR

Part.Size	0.2 (mg)	0.3 (mg)

N for the mean = 18

Analysis of variance tables for determination of significant differences (at $P<0.05$) among: Days, Ceramic Replicates, and Interaction between Days and Ceramic Replicates during the Delivery of Gamma Globulin (mg) by ALCAP ceramics in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C.

Particle Size 35-45 um

Factors	S.S.	D.F.	M.S.	P.	F-ratio
Days	341.231	6	56.872	0.16×10^{-10}	216.961 *
Cer. Rep.	0.048	2	0.024	0.732	0.313 NS
Days x Cer. Rep.	3.146	12	0.262	0.28×10^{-3}	3.439 *
Error	8.003	105	0.076		

Particle Size 45-60 um

Factors	S.S.	D.F.	M.S.	P.	F-ratio
Days	355.665	6	59.278	0.16×10^{-13}	689.125 *
Cer. Rep.	0.310	2	0.155	0.078	2.619 NS
Days x Cer. Rep.	1.032	12	0.086	0.154	1.453 NS
Error	6.215	102	0.059		

Particle Size 60-75 um

Factors	S.S.	D.F.	M.S.	P.	F-ratio
Days	401.098	6	66.850	0.2×10^{-12}	451.603 *
Cer. Rep.	0.026	2	0.013	0.829	0.188 NS
Days x Cer. Rep.	1.776	12	0.148	0.010	2.149 *
Error	7.232	105	0.069		

S.S. = Sum of squares
D.F. = Degrees of freedom
M.S. = Mean of squares

* = Significant at $P<0.05$
NS = Not significant at $P<0.05$

Appendix 4-C

Comparison Among Means by Student-Newman Keuls Test on Amount of Insulin (mg) Delivered by the Different Particle Sizes of ALCAP Ceramics in Continuous Flow-Through System of Phosphate Buffered Saline (pH 7.4) at 37°C on each day for a period of seven days.

DAYS	MEANS OF AMOUNT DELIVERED		
1	3.5^{s}	3.5^{m}	5.6^{L}
2	5.4^{s}	5.5^{m}	6.4^{L}
3	6.5^{s}	7.6^{m}	9.5^{L}
4	8.4^{m}	8.5^{s}	9.6^{L}
5	7.4^{L}	8.3^{m}	8.5^{s}
6	5.5^{m}	6.5^{s}	6.5^{L}
7	4.4^{L}	5.5^{s}	6.5^{m}

s = Particle size 35-45 um
m = Particle size 45-60 um
L = Particle size 60-75 um

APPENDIX 5

Preparation of Phosphate Buffer Saline (pH 7.4)

For 15 Liters

1. Dissolve 122.6 grams NaCl in about 500 ml distilled water in a 1000 ml beaker.
2. Add 85.7 ml, .5 M $NaH_2PO_4.H_20$ (sodium phosphate-monobasic).
3. Add 222.9 ml, of .5 M NA_2HPO_4 (sodium phosphate-dibasic).
4. Add 0.1 g of Merthulate to prevent contamination.
5. Dissolve completely and transfer to 15 liter contained. Dilute to 15 liters with distilled water.
6. Check pH.
7. Store in refrigerator.
8. Adjust pH to using the monobasic solution if it is above 7.4 or the diabasic solution if it is below 7.4.

Scanning Electron Microscopy (Method of Khot, et al., 1980)

A JEOL JSM-U3 scanning electron microscope (SEM) with a resolution of 200°A was used to magnify the cracked surface of the ceramic 250 X. A conductive coating of a 60% Ag - 40% Pd allo was vapor deposited on a piece of a cracked ceramic surface before they were used to deliver the various polypeptides.

Lowry Determination of Protein

1. Prepare the following reagents.

Reagent A_1: - $NA_2C_4H_4O_6 \cdot 2H_2O$ (sodium tartrate-----2 grams.

Add distilled water to make 100 ml.

Reagent A_2: - $CuSo_4 \cdot 5H_2O$ (copper sulfate)-----1 gram.

Add distilled water to make 100 ml.

Reagent A_3: - Na_2CO_3 (sodium carbonate)-----20.0 grams.

Add 0.1 N NaOH to make 1 liter.

Reagent A: -

To prepare Reagent A, mix the following quantities together in the following sequence. Reagent A should be prepared new for each test and not kept more than 24 hours.

A_1	0.5 ml
A_2	0.5 ml
A_3	50.0 ml

Reagent B: - Phenol reagent (Folin Ciochlteau) comes bottled in a 2N solution for the test. Dilute this to 1N.

Note: Use volumetric flasks to prepare reagents A_1, A_2 and A_3.

Preparation of Standard Curve

Procedure:

1. To prepare protein standard solution, mix a stock solution of Bovine Serum Albumin in distilled water at a concentration of 700 microgram/ml and use the following dilutions for standard curve.

Test Tube	#1	#2	#3	#4	#5
Conc of BSA (ug/ml)	0	35	70	175	350
Water	1ml	0.95ml	0.9ml	0.75ml	0.5ml
BSA	0ml	0.05ml	0.1ml	0.25ml	0.5ml

2. To run the assay, mix 1 ml of each BSA standard with 5.0 ml of Reagent A and let this mixture stand at room temperature for 10 minutes. To this, add 0.5 ml of Reagent B and immediately mix in a vortex mixer. Allow all samples to stand at room temperature for 30 minutes. Record optical density on a Bausch and Lomb Spectronic 20 at 700 nm. Be sure to use the right lamp and the red filter. Read the optical density of each tube and plot it in units of ug/ml of BSA.

Preparation of Polypeptide Standards

(1) Insulin

Crystalline insulin from bovine pancreas (No. I-5500) yielded hazy solution in distilled water at 10 mg/ml. Therefore, it was dissolved in acid medium (pH 3) as per instruction by Sigma Chemical Company. The concentration used for conducting this research was 100 mg/ml.

(2) Gamma Globulin, BSA, and Chymotrypsinogen-A

To prepare the above polypeptide standards (100 mg/ml) dissolve 10 mg of the polypeptide in a 100 ml of PBS using very slow magnetic stearer (about 20 rpm) and a sterilized beaker, for about 4 hours or until all the floating powder becomes suspended.

Appendix (6)

Fabrication of Ceramics
(Procedure of Khot, 1979)

Final shape of the ceramic was obtained by following the step by step procedure as given below:

(1) 58 grams of Aluminum oxide, 42 grams of calcium oxide, and 20 grams of phosphorous pentoxide were weighed and placed in a bottle. The material was then placed on a ball mill roller for 10 mins. to obtain thorough mixing.

(2) Ceramic crucibles (Norton Company) were filled with the above mixture in a hood. The mixture was loosely filled so that the crucibles could be used again.

(3) The furnace (Leco) was preheated at 316°C and crucibles containing the mixture were fixed at 1315°C for 10 hours. The furnace was turned off after 10 hours and allowed to cool for 24 hours.

(4) The warm crucibles were removed from the furnace one at a time. The calcined material was ground to a powder state using a mortar and pestel.

(5) The ground powder was placed on a Tyler sieve stack. The following mesh sieves were used to separate various particles. The sieves were used to separate various particles. The sieves were stacked on a shaker (Ro-Tap-Testing Sieve Shaker) and the contents were shaken for 10 minutes. The sieve sizes were stacked as shown in the table below.

(6) Powders of various particles size ranges were stored in capped bottles. The bottles were labelled with particle size range and rate of calcining.

Table:

	Stack Placement	Sieve Size	Particle Size (u)
Top	1	70	210
	2	100	147
	3	120	125
	4	170	88
	5	200	75
	6	250	60
	7	325	45
	8	400	35
	9	Catch Pan	35

(7) The particle size powders ranges 35-45 u, 45-60 u, and 60-75 u were used to prepare the ceramic cylinders. 3.3 grams of each of the above calcined-particle-size-range powders were added to 0.1 grams of polyvinyl alcohol powder (147 u) and mixed thoroughly. Polyvinyl alcohol acts as a binder as well as a die lubricant. This mixture was put into a cylindrical tungeston die (2.0 cm long, 1.3 cm outside diameter and 0.5 cm inside diameter) and pressed hydraulically in a Clifton Hydraulic Press for five seconds at approximately 11,000 psi.

(8) The furnace was preheated at 316°C and all ceramic cylinders were placed into it for final sintering immediately after pressind. All the ceramics were fired at 1425°C for 24 hours. After 24 hours the furnace was turned off and allowed to cool for 24 hours. The

ceramics then stored in a vacuumed dry container to be used for delivering gamma globulin, bovine serum albumin, chymotrypsinogen, and insulin in a continuous flow-through system of phosphate buffered saline (pH 7.4) at 37°C for a period of 7 days.

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